

ASSESSMENT OF FACTORS INFLUENCING TEXAS WILD-RICE (*ZIZANIA TEXANA*) SEXUAL AND ASEQUAL REPRODUCTION

2004 FINAL REPORT

Prepared for

Edwards Aquifer Authority
1615 North Saint Mary's Street
San Antonio, Texas 78215



San Marcos National Fish Hatchery and Technology Center
500 East McCarty Lane
San Marcos, Texas 78666



Lady Bird Johnson

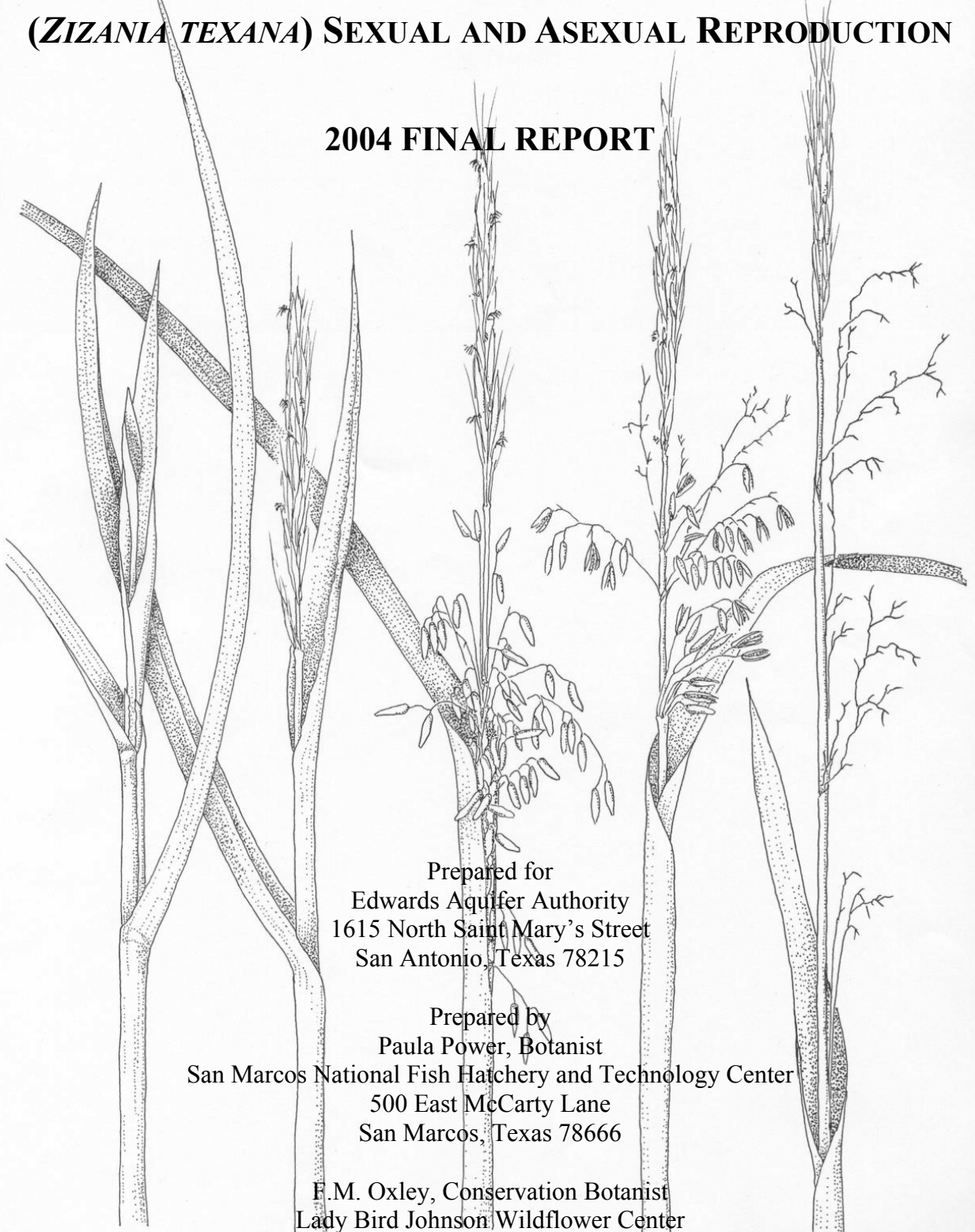
Wildflowercenter

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4801 La Crosse Avenue
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EXECUTIVE SUMMARY

The following document reports on activities undertaken between January 2000 and June 2004 as outlined in the Joint Funding Agreement 00-64-AS between the Edwards Aquifer Authority and the U.S. Fish and Wildlife Service, San Marcos National Fish Hatchery and Technology Center. The three-year study was designed to identify factors that influence sexual and asexual reproduction in Texas wild-rice (*Zizania texana*). Four manipulative studies, a field survey, and a compilation of existing seed harvest data (1999 – 2003) were used to examine the characteristics of pollen viability and dispersal, stigma viability and receptivity, ability to self-pollinate, seed production and quality, minimum distances for successful pollination within and among stands, and asexual output.

Zizania texana is a wind pollinated grass, producing separate male and female flowers in a panicle-type inflorescence at the terminal end of emergent stems called culms. Male flowers, or anthers, produce abundant pollen which is released and carried by the wind to stigmas of female flowers (pollination). Torpedo-shaped, olive colored seeds ripen and fall from the panicle into the water. If seeds drop to the river bottom in a suitable location, they will germinate and grow into a new plant. If seeds float, however, they are carried by the current and their fate is unknown.

Zizania texana is capable of reproducing sexually, and does so by utilizing a mixed pollination strategy (Chapter 1). Cross pollination (xenogamy) is the primary means of pollination, while between-inflorescence pollination plays a second, supporting role. Within-inflorescence pollination appears to play little or no part in the pollination strategy. Pollen viability is limited and may present an obstacle to successful sexual reproduction in the wild. *Zizania texana* pollen is viable for only a short period of time and viability begins to decrease 10 minutes after the pollen has been released from the anthers; pollen is completely dead one hour after release from anthers. Stigmas remain receptive for approximately three days, after which receptivity begins to decline. Stigmas are least receptive eight days after they appear.

Most wind pollinated species release pollen throughout the day. Typically, pollen is released early in the day, usually before noon, when temperatures are warm and humidity is low. In contrast, *Z. texana* releases most of its pollen during the pre-dawn hours, between 0200 hours and 0400 hours, when temperatures are cooler and humidity is higher relative to daytime temperatures and humidity. There also is a secondary release around 0900 hours (Chapter 2).

To study pollen dispersal, pollen traps made from glass slides with petroleum jelly applied to the surface, were placed at intervals from a source of mature flowers for 24-hour periods. Pollen grains on traps were considered a measure of the distance pollen travels and the relative amount of pollen available to pollinate receptive stigmas. There was significantly more pollen on traps within 0.75 m of the pollen source and very little to no pollen from 0.75 m to 12.0 m from the pollen source. Furthermore, there was

significantly more pollen on traps when more than 40 panicles were releasing pollen compared with fewer than 40 panicles (Chapter 2).

When grown in captivity under conditions simulating its San Marcos River habitat, *Z. texana* is reproductive every month of the year (Chapter 3). However, there are peak reproductive periods. Using data from 1999 through 2003, the greatest number of inflorescences and seed produced was March – June. The month with the highest number of seeds per inflorescence was April (26.2 seeds per inflorescence) while the annual mean was 16.6 seeds per inflorescence.

There was variation in the weight of seeds produced both seasonally and when comparing seeds that sink or float when dropped in water. Of seeds that sink, those with the greatest mean weight (0.015 g per seed) were produced in March, April, May, and December. Those with the least mean weight (0.011 g per seed) were produced during August and January. Of seeds that float when dropped in water, those with the greatest mean weight (0.012 g per seed) were produced during May. Those with the least mean weight (0.008 g per seed) were produced during January, March, August, and December. Whether seeds sink or float may have ecological significance because seeds that sink have significantly higher percent germination compared to seeds that float (Chapter 4) and a larger seed will provide more nutrients to the developing seedling.

There was also seasonal variation in the proportions of seeds that either sink or float. During April and May 79% and 77%, respectively, of all seeds produced sank when dropped in water. During the rest of the year, more than half of all seeds produced floated when dropped in water.

A field survey of randomly selected *Z. texana* plants documented culm density in *Z. texana* stands and also the condition of culms (e.g., if culms were emergent or damaged) (Chapter 5). The mean number of culms per 0.065 m² was 8.03. This is about the area occupied by a single individual. One percent of culms in the survey were in the seed-producing stage. Forty-five percent were either submersed and developing, or emergent and potentially reproductive. Fifty-four showed some impediment to reproduction. With over half of all culms exhibiting an impediment to reproduction, the survey data support the prevailing opinion that extrinsic factors are responsible for reproductive failure in *Z. texana*.

Asexual reproduction is a component of the reproductive biology of *Z. texana*. In a 20-week study, 25 of 25 study plants produced reproductive culms. During this same 20-week period 22 of 25 plants produced asexual tillers. Asexual tillers arise from nodes along culms. Tillers appear first as new leaves at a node followed by the development of roots. Eventually the tiller will root adjacent to the parent plant or the tiller will detach from the node, drift in the current and potentially root in a new location. Successful recruitment by tillers is assumed but there are no data to verify the occurrence or rate of recruitment.

The combined information in the chapters of this report creates a description of the reproductive biology of *Z. texana*. Pollen is released during the relatively cool morning hours (between 0200 and 0400) and is short lived (<10 min). Pollen from one inflorescence does not pollinate the female flowers of the same inflorescence; therefore, for successful pollination to occur, the maturation of panicles must be staggered. This promotes outcrossing (xenogamy) and novel combinations of alleles. Pollen often travels less than 1 m and in this study, significantly more pollen is distributed when there are more than 40 panicles releasing pollen. It can be inferred that successful pollination would occur in large stands with less than 1 m gaps between stands. Gaps greater than 1 m would limit gene flow between stands. Based on the field survey, few individual *Z. texana* plants grow in isolation and most plants have other *Z. texana* as neighbors. This is to be expected in a clonal, wind pollinated species. Results from Chapters 2 and 5, together with TPWD's monitoring of the wild population, suggest that a critical impediment to sexual reproduction occurs at the pollination stage. Fragmentation of stands, gaps between stands, and damaged culms all contribute to failure at the pollination stage. The notable exception to reproductive failure occurs in Sewell Park, where seeds were produced from 1998 to 2004, where the stands are larger and the gaps between stands are smaller relative to the rest of the population.

POLLINATION STRATEGY

F. M. Oxley, Jackie Pendergrass, and Paula Power

Abstract

One of three North American species of *Zizania*, *Z. texana* is a perennial, aquatic macrophyte endemic to the upper 7.62 km (river km) of the San Marcos River. Historically abundant throughout the upper San Marcos River, in Spring Lake, and along the adjacent irrigation ditches, *Z. texana* significantly declined between 1940 and 1967. Among a number of factors, the decline has been attributed to reproductive failure. Although the production of inflorescences has been observed in the wild, the plants still rarely set seed. A bagging experiment examined the ability of *Z. texana* to self-pollinate, specifically the ability to self-pollinate by 1) flowers on the same inflorescence; and, 2) flowers on different inflorescences produced by the same plant.

Pollen viability was tested by germinating pollen samples in 20% Brewbaker-Kwack medium. Seven subsamples, at ten-minute intervals (0, 10, 20, 30, 40, 50, 60), were tested and percent germination calculated. Pollen viability decreased significantly ten minutes after release from the anthers.

Color-coded stigmas were collected over a period of seven days and tested for receptivity using Baker's solution. Progressively fewer stigmas were receptive each day for eight days. In the bagging study, all treatments produced seeds and within-panicle self-pollination treatments produced significantly fewer seeds than control plants. It is clear that *Z. texana* is capable of reproducing sexually and does so by utilizing a mix of cross-pollination and self-pollination.

INTRODUCTION

Pollination is defined as the transfer of pollen from the anthers of one flower to the stigma of another flower within the same species (Kearns and Inouye, 1993; Mauseth, 1991; Northington and Goodin, 1984). It is the critical first step in a sequence of events, including fertilization and seed set, that leads to successful sexual reproduction in plants (Lyons et al. 1989). Without successful pollination, sexual reproduction will not occur.

Pollination in flowering plants can be categorized into two broad strategies: cross-pollination and self-pollination (Northington and Gooding, 1984). The flowers of cross-pollinating (xenogamous) species are pollinated by pollen transferred from other individuals in the same species. Cross-pollinated species have access to larger gene pools and, as a result, genetic variability within the species is assured.

Unlike cross-pollinating species, self-pollinated species (autogamous) are pollinated by pollen from within the same flower. Flowers pollinated by pollen from another flower on the same plant (geitonogamous) are also considered to be self-pollinated (Northington

and Goodin, 1984). This pollination strategy has basically the same results as asexual reproduction in that it does not involve large gene pools, has no possibility of introducing new genes from outside the parent plant, and leads to a decrease in the genetic variability within the species (Mauseth, 1991; Northington and Goodin, 1984).

Pollen viability and stigma receptivity also play a role in a species' reproductive success. Pollen is an essential component for sexual reproduction and gene flow (Kearns and Inouye, 1993). It is crucial that pollen is viable once it is deposited on receptive stigmas. Dead pollen cannot germinate and the reproductive process stops. Pollen viability can be affected by a number of factors. Viability can vary between individuals within a given population. Differences in the quality of the plants' environment (Young and Stanton, 1990) and pollen age (Thomson and Thomson, 1992) also contribute to pollen viability.

In order for successful reproduction to occur, pollen must not only be moved from one flower to another, but it also must be deposited on receptive stigmas (Kearns and Inouye, 1993). If pollen is deposited before or after stigmas are receptive, it may not germinate and reproductive failure will occur. Stigma receptivity also affects a number of other reproductive processes including the rate of self-pollination, successful pollination at different stages of floral development, and the relative importance of various pollinators (Galen et al. 1986).

Abiotic pollination by wind (anemophily) is found predominately within the grasses (Poaceae) and grass-like species (Cyperaceae and Juncaceae) (Erickson and Buchman, 1983). Wind pollinated species exhibit several distinguishing floral characteristics: numerous small flowers, separation of the sexes, aggregation into dense inflorescences, copious amounts of lightweight, smooth-walled pollen, and large, feathery stigmas (Faurey et al. 1997; Mauseth, 1991; Northington and Gooding, 1984). Large numbers of flowers in dense inflorescences increases the chances of pollination occurring. Stamens hang from long, flexible filaments, exposing the anthers to passing winds. Massive amounts of pollen are produced, much of which never reaches its destination. The large feathery stigmas present expanded surface areas to wind currents for the capture of airborne pollen. Wind pollinated species are generally found growing in dense populations in open areas (Mauseth, 1991; Northington and Goodin, 1984).

One of three North American species of *Zizania*, *Z. texana* is a perennial, aquatic macrophyte endemic to the upper 7.62 river km of the San Marcos River (U.S. Fish and Wildlife Service, 1978; Terrell et al. 1978; Gould, 1975). A member of the Poaceae (Grass Family), *Z. texana* produces long, ribbon-like submersed leaves throughout the year. Emergent culms are produced during the spring, summer, and fall, root at the nodes, and bear terminal inflorescences (Power, 1997; Terrell, et al., 1978; Gould, 1975). The development of the inflorescences is similar to that of *Z. aquatica* L. as described by Weir and Dale (1960). The inflorescences are composed of both pistillate and staminate flowers on separate spikelets. Floral development is protogynous with pistillate flowers developing first and positioned at the top of the inflorescence. Staminate flowers develop later than the pistillate flowers and are positioned below pistillate flowers. Feathery

stigmas are produced over several days followed by the production of anthers and the release of pollen (Power, 1997).

Historically, *Z. texana* was described as being abundant throughout the upper San Marcos River, in Spring Lake, and along the adjacent irrigation ditches (Silveus, 1933). In 1967, Emery found only one plant growing in Spring Lake, no plants in the first 0.8 km of the river below the dam, and only scattered stands after that.

At the time of his initial survey of the species, Emery (1967) also noted that *Z. texana* was reproducing primarily by vegetative means. Ten years later, he revisited the site and observed that the plant still was not reproducing sexually in the river nor had there been an increase in the population through asexual reproductive mechanisms (Emery, 1977). This continued decline of *Z. texana* in the wild, combined with its lack of sexual reproduction, led to its listing as an endangered species in 1978 by the U.S. Fish and Wildlife Service (U.S. Fish and Wildlife Service, 1978).

Emery and Guy's (1979) study of reproductive ability and embryo development of *Z. texana* showed that the species is capable of sexually reproducing and producing viable offspring. There has been some question about *Z. texana*'s ability to self-pollinate. According to Weir and Dale (1960), members of the genus *Zizania* are incapable of self-pollination, while Terrell et al. (1978) noted the use of seeds produced from self-pollination in germination studies, indicating that self-pollination is possible for *Z. texana*. Neither of these papers indicated whether or not self-pollination was within-panicles or between-panicles. Power's study (1997) examined the species' ability for self-pollination and the results of that study indicated that *Z. texana* is incapable of within-panicle self-pollination, although there was some concern that the bagging method may have affected fertilization and seed set.

Although emergent inflorescences have been observed in the wild population, (Emery and Guy, 1979; Terrell et al. 1978; Emery, 1977, 1967), the plants still rarely set seed in the San Marcos River. This, together with its limited and fragmented distribution, makes *Z. texana* vulnerable to continued decline and loss of genetic variability in the wild.

The objective of this study was to further examine self-pollination in *Z. texana* and to collect baseline data on pollen viability and stigma receptivity.

METHODS AND MATERIALS

The self-pollination, pollen viability, and stigma receptivity studies took place at the San Marcos National Fish Hatchery and Technology Center (NFHTC) located in San Marcos, TX (Figure 1-1).



Figure 1-1. National Fish Hatchery & Technology Center, San Marcos, Texas.

Two experimental raceways (17 m x 1.5 m x 0.9 m) were set up. Each raceway was divided in half lengthwise using either a series of 2.4 m x 0.6 m 14 m sheets of 6 mm thick Plexiglas or a cinderblock divider (Figure 1-2).



Figure 1-2. Experimental raceway setup.

Pumps were placed at intervals on either side of the dividers to provide constant water movement. Water was pumped from an on-site well at a rate of 3.8 l/minute and a depth of 0.75 m.

Study plants were grown from seeds collected during 2001 from the *Z. texana* conservation population located at Texas State University (TSU) in San Marcos, TX. Seeds were placed in wet paper towels and sealed in Ziploc® style plastic bags. The seeds were cold stratified at 3°C for a period of 3 - 6 months. Following stratification, seeds were placed in glass vials (3 – 5 seeds/vial) containing 3 – 4 ml tap water. Vials were capped to create the anaerobic conditions required for germination (Power and Fonteyn, 1995) and placed in an environmental chamber set at 12 hours light/12 hours dark and 21°C $\pm$ 1°C for one week. After one week, the vials were uncapped, topped off with tap water, and placed back in the environmental chamber for an additional week. Germinated seeds were removed from the vials, placed in a cup of tap water, and set in a sunny spot for an additional week.

Seedlings were planted in 12 cm x 11cm diameter plastic pots lined with plastic bags to retain the soil and filled with 1000 cm³ of a 1:1 potting mixture of sand and site-collected soil that had been heated to 65°C for 24 hours. Seven grams of Osmocote™ time-released fertilizer were added to the soil mixture for additional nutrition. Five to six seedlings were planted in each pot.

As the seedlings matured, individual seedlings were transplanted into pots lined with plastic bags and filled with 3500 cm³ of the potting mixture. Approximately 300 plants were randomly assigned a number and placed in the raceways. In addition to seedlings, vegetatively grown tillers were also potted and randomly placed in the raceways. Each raceway had a mix of seedlings and tillers.

The ability of *Z. texana* to self-pollinate was examined from 15 May – 17 August 2002. Pollination strategies were examined using five manipulative treatments and one control, each with ten replicate plants (60 plants total). Inflorescences from each plant were bagged (Figure 1-3) as they emerged using 100% acetate fabric bags (73 cm x 22 cm) with plastic windows to allow observation of developing inflorescences (*pers. comm.*, Carol Kearns, University of Colorado; Zhou et al. 1999; Nerd et al. 1998; Ortega-Olivencia and Devesa, 1998; Froborg, 1996). Bagged inflorescences were treated using one of the following pollination techniques:

1. **Within-panicle self-pollination (WIG):** individual inflorescences were bagged and allowed to develop to maturity.
2. **Within-panicle hand self-pollination (WIHG):** individual inflorescences were bagged and hand pollinated with a paintbrush using pollen collected from the same inflorescence. Anthers were removed daily to prevent self-pollination. Hand pollination continued until the stigmas were no longer present and/or the anthers were finished releasing pollen. The inflorescences were allowed to develop to maturity.

3. **Between-panicle self-pollination (BG):** two inflorescences produced by the same plant were bagged together as they emerged. As flowers developed, one inflorescence was emasculated (leaving the pistillate flowers) and pistillate flowers were removed from the other inflorescence (leaving the staminate flowers). The two inflorescences remained bagged together and allowed to pollinate in the bag. Inflorescences were then allowed to develop to maturity.
4. **Between-panicle hand self-pollination (BHG):** one inflorescence was bagged as it emerged. Pollen from another flower produced by the same plant was brushed on the stigmas of the bagged inflorescence using a paintbrush. Hand pollination continued in this manner until the stigmas were no longer present. The bagged inflorescence was emasculated, as the stamens appeared to prevent self-pollination.
5. **Control (C):** inflorescences were allowed to mature without bagging.
6. **Emasculate (Emas):** inflorescences were emasculated to prevent self-pollination and allowed to mature without bagging.



Figure1-3. Acetate bags used in pollination study.

Inflorescences in all treatments were allowed to set seed. Inflorescences of both control and emasculate treatments were bagged as seeds began to develop using muslin cloth bags to prevent their loss. The inflorescences of all other treatments remained bagged until seeds were harvested. Inflorescences were harvested seven to ten days after seeds were first observed. The number of inflorescences setting seed were counted and recorded. Data were analyzed using a Chi-square 2 x 6 contingency table ($\alpha = 0.05$) and non-parametric Tukey multiple comparison test ($\alpha = 0.05$).

Pollen viability was examined from 15 May – 17 August 2002. To determine the length of time that pollen is viable, ten inflorescences in the staminate stage (Fig. 1-4) were randomly selected from the experimental population.



Figure 1-4. Stages of floral development of *Zizania texana*. A. Emergent stage are the days between the time when the first leaf tip breaks the water surface until the first pistillate spikelet appears from the leaf sheath; B. Pistillate stage are the days between the appearance of the first pistillate spikelet and the appearance of the first anther; C. Staminate stage are the days between the appearance of the first anther and the dehiscence of the first anther; D. Full panicle development are the days between the first and last anther dehiscence; and E. Seed development stage are the days between the last anther dehiscence and last seed abscission.

Pollen was collected in the morning between 6 and 7 a.m. because this was the time of day with the least amount of wind and pollen was easily collected. Each inflorescence was sampled multiple times to have adequate numbers of independent pollen samples. Pollen was collected from a single inflorescence beginning the first day that anthers dehisced and continued until all anthers were dehisced. Open anthers from which pollen was collected were removed after sampling each morning to ensure that “fresh” pollen was collected each day.

Each inflorescence was sampled by tapping the entire inflorescence into a Petri dish containing a glass slide. A drop of 20% Brewbaker-Kwack medium (1963) was placed on the pollen collected on the slide (time = 0). The remaining pollen in the Petri dish was divided into 6 sub-samples. A drop of Brewbaker-Kwack medium was added to each sub-sample at 10-minute intervals (10, 20, 30, 40, 50, and 60). Following the addition of Brewbaker-Kwack medium, each sub-sample was allowed to incubate at room temperature for 15-30 minutes (Khatun and Flowers, 1995).

Using a compound microscope at 100X, germinated and ungerminated pollen grains were counted on each slide. A minimum of 100 pollen grains was counted for each time interval. Pollen grains were considered germinated when pollen tube length was greater than or equal to half the diameter of the pollen grain (Rodriguez-Riano and Dafni, 2000; Lansac et al. 1994).

Percent germinated pollen was calculated in the following way:

$$\% \text{ germinated pollen} = \frac{\# \text{ of germinated pollen grains}}{\text{total \# of pollen grains counted}}$$

Data were analyzed using a Chi-square 2 x 7 contingency table ($\alpha = 0.05$) and non-parametric Tukey multiple comparison test ($\alpha = 0.05$).

To determine the length of time stigmas were receptive, stigmas were tested using Baker’s solution, which detects the presence of alcohol dehydrogenase (Dafni and Maues, 1998).

Twenty-one inflorescences were bagged as they emerged during the pistillate stage of floral development. Stigmas were color-coded as they developed over a period of seven days (black = day 2, brown = day 3, purple = day 4, green = day 5, orange = day 6, blue = day 7, red = day 8). Day 1 stigmas were collected and tested on the day they first appeared (Shafer et al. 2000; O’Brien, 1996). Color-coded stigmas were collected on the appropriate day, placed in a drop or two of Baker’s solution on a glass slide in a Petri dish containing moist filter paper, covered, and allowed to incubate for 30-40 minutes (Dafni and Maues, 1998). Stigmas were examined under a dissecting microscope. Stigmas were considered receptive if any part of the stigma was stained purple to tannish-brown (Figure 1-5).



Figure 1-5. Feathery stigma of *Zizania texana*. Receptive stigmas stained purple to tannish-brown.

For data analysis, receptive stigmas were assigned a value of 1 and non-receptive stigmas were assigned a value of 0. Data were analyzed using Cochran's Q test, which is used to analyze dichotomous nominal-scale data with repeated measures as described in Zar (1999). Multiple comparison analysis was conducted using a non-parametric Tukey multiple comparison test ($\alpha = 0.05$).

RESULTS

Ten replicates each were used for the control (C), emasculate (Emas), and within-panicle self-pollination (WIG) treatments. Nine replicates each were used for within-panicle hand pollination (WIHG), between-panicle self-pollination (BG), and between-panicle hand self-pollination (BHG) treatments. Fewer replicates were used for the WIG, BG, and BHG treatments due to the number of inflorescences available for the crosses. Control inflorescences produced significantly more seed than WIG inflorescences ($p < 0.05$). Inflorescences setting seed in all other treatments were intermediate between controls and WIG (Figure 1-6).

A total of 76 pollen samples were collected for the 0 minute time interval and 71 samples were collected for the remaining time intervals (10, 20, 30, 40, 50, 60 minutes). Pollen viability at time = 0 was significantly greater than all other time intervals, and 83% of samples contained germinated pollen. No significant difference was observed between the other time intervals (Figure 1-8). The percentage of samples with germinating pollen significantly declined in the remaining time intervals with no significant difference between remaining time intervals (Figure 1-7).

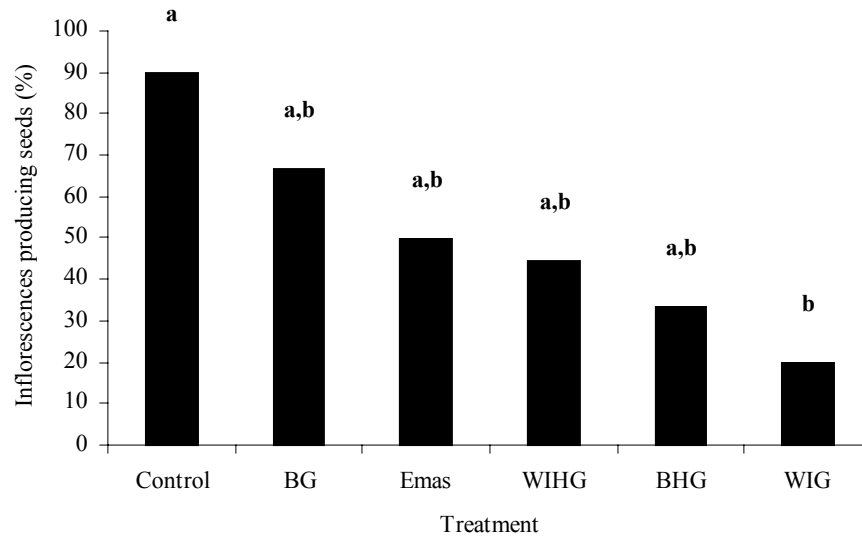


Figure 1-6. Percentage of inflorescences producing seed for each pollination treatment (n = 10). Treatments were Control (no treatment), Emas (anthers removed), between-panicle self-pollination (BG), between-panicle hand self-pollination (BHG), within-panicle self-pollination (WIG), and within-panicle hand self-pollination (WIHG). Values of non-parametric Tukey's multiple comparison tests are indicated by letters above columns. Same letters indicate no significant difference at $\alpha = 0.05$ and different letters indicate significant difference at $\alpha = 0.05$.

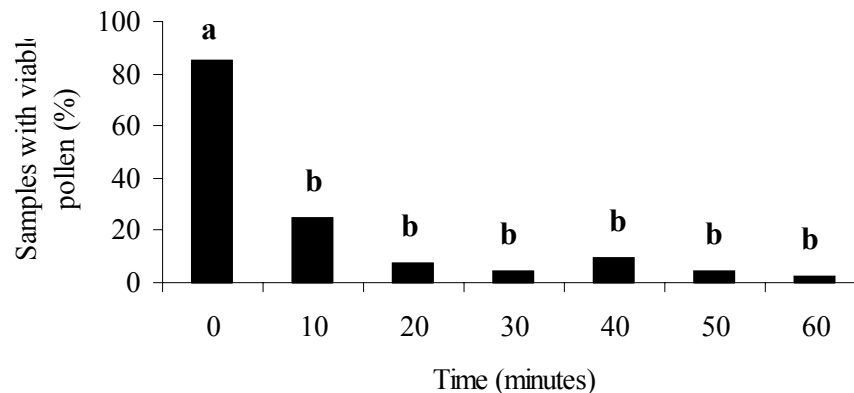


Figure 1-7. Percentage of samples with germinated pollen over subsample time intervals. Values of non-parametric Tukey's multiple comparison tests are indicated by letters above columns. Same letters indicate no significant difference at $\alpha = 0.05$ and different letters indicate significant difference at $\alpha = 0.05$.

Stigmas were sampled from a total of 21 plants. Cochran's Q-test indicated significant differences in receptivity over time. As the pistillate stage progressed, fewer receptive stigmas were present (Figure 1-8).

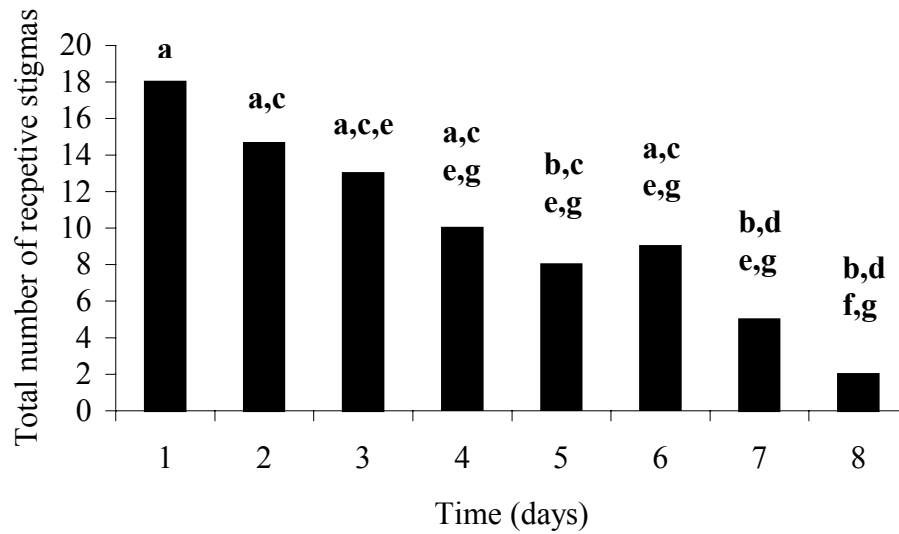


Figure 1-8. Receptivity of stigmas sampled from 21 randomly selected *Zizania texana* plants over an eight-day period.

DISCUSSION

Like most anemophilous species, *Z. texana* pollen must wait for wind currents to remove it from open anthers and deposit it on receptive stigmas. Once the anthers have opened, pollen begins to dessicate. The longer pollen remains in open anthers before being transported by wind currents, the more dessicated it will be once it becomes airborne. Pollen viability varies from species to species. While the pollen viability of some species declines over a period of several days (Pacini et al. 1997; Tangmitcharoen and Owens, 1997), others decline within a matter of minutes (Khatun and Flowers, 1995). The pollen of *Z. texana* appears to be very short lived. Pollen viability significantly declined ten minutes after being released from the anthers and was completely dead an hour after being released from the anthers.

A number of factors, including dessication, humidity, and temperature, can affect pollen viability (Luna et al. 2001; Pacini et al. 1997; Springer et al. 1989). Of these, dessication may be a contributing factor in the loss of *Z. texana* pollen viability. While the pollen of many flowering plants is usually dessicated to some extent by the time it reaches a receptive stigma (Heslop-Harrison, 1979), most are able to rehydrate once they land on a receptive stigma. This may not be the case for *Z. texana*.

Stigma receptivity is a critical step in determining successful pollination (Dafni and Maues, 1998). While, in some species, stigma receptivity increases with age (Kalisz et al. 1999; Galen et al. 1986), this does not appear to be the case in *Z. texana*. Stigmas were most receptive during the first three days and receptivity began to decline on the fourth day. By days 7 and 8, many of the stigmas had fallen off the pistillate flower.

The decline of *Z. texana* stigma receptivity over time, combined with its limited pollen viability, may be contributing factors to reproductive failure in wild stands that are highly

fragmented and widely spaced. Floral development of *Z. texana* indicates that outcrossing is the primary method of pollination in this species (Power, 1997; Emery and Guy, 1979) and this study supports that conclusion. The limited number of within-panicle self-pollinated inflorescences setting seed suggests that *Z. texana* is incapable of effective self-pollination within a single inflorescence.

Barriers to within-panicle self-pollination may be timing of floral development and/or physical or chemical barriers to pollen germination. Very limited seed set occurred in the within-panicle crosses, indicating the timing of floral development may be the primary barrier to self-pollination in *Z. texana*. According to Power (1997), there are three stages of floral development in *Z. texana*: a pistillate stage, staminate stage, and full panicle development. The pistillate stage, lasting approximately two days, occurs between the emergence of the first pistillate spikelet and the exertion of the first anther. The staminate stage lasts approximately three days and begins with the exertion of the first anther and ends with the dehiscence of the first anther. Full panicle development is the number of days, approximately four, until all the anthers have dehisced. Full floral development takes approximately nine days to complete (Figure 1-4).

For within-panicle self-pollination to occur, anthers must be releasing viable pollen at the same time stigmas are at their most receptive. This does not appear to be the case for *Z. texana*. The results of this study indicate that more stigmas are receptive during the first three days of floral development (pistillate stage) with receptivity beginning to decrease on day four. This coincides with the beginning of the staminate stage. By the end of the staminate stage (day five), there are significantly fewer receptive stigmas (2.3 times less) than there were on day one and the potential for successful within-panicle self-pollination occurring is significantly reduced. By the time full panicle development occurs and all anthers are dehisced, stigmas are no longer receptive and the opportunity for within-panicle self-fertilization is past.

The floral development of *Z. texana* favors between-panicle self-pollination, because the possibility chances of overlapping pistillate, staminate, and full panicle stages is increased. Between-panicle crosses produced 20% seed set, slightly less than that of the controls (22%). This suggests that self-pollination between related inflorescences may be just as effective as cross-pollination and may contribute to *Z. texana*'s reproductive strategy.

Emasculated inflorescences produced the same seed set as the within-panicle self-pollination treatments. This is interesting because it was expected that the emasculated panicles would have approximately the same seed set as the controls. Emasculated plants appeared to be less robust than plants that were not emasculated. It is possible that removing the anthers may have affected the overall health of the emasculated plants and reduced their reproductive ability. More study is needed on the effect of emasculation on seed production.

The results of this study indicate that *Z. texana* employs a mixed pollination strategy. While cross-pollination is the primary means of pollination, between-panicle self-

pollination appears to be a second, supporting means of pollination. Within-panicle self-pollination appears to play little or no part in the reproductive strategy of *Z. texana*.

Historically, *Z. texana* was described as being abundant throughout the upper San Marcos River, in Spring Lake, and along the adjacent irrigation ditches (Silveus, 1933) and photos taken during this time show large stands with many inflorescences maturing at the same time. As a result, large amounts of pollen were available and had to travel very short distances before coming into contact with receptive stigmas. Pollen longevity in this type of system would be unimportant (Pacini, et al. 1997).

Today, the population is highly fragmented with large gaps between stands. Flowering is sporadic and spotty within each stand. In this scenario, pollination appears to be a major impediment to reproductive success for *Z. texana*. The lack of sufficient numbers of flowering inflorescences within stands with receptive stigmas and dehiscing anthers at any one time means that not enough pollen is being released during the time stigmas are receptive and fewer receptive stigmas are available when pollen is abundant. The large gaps between stands means that pollen must travel farther to reach receptive stigmas and may be dehydrating and dying before it reaches its target.

It is clear that *Z. texana* is capable of reproducing sexually. Plants capable of self-pollination have a reproductive advantage in that they can reproduce when outcrossing is difficult, disadvantageous, or impossible (Lloyd and Schoen, 1992). This combination of pollination strategies may help *Z. texana* overcome the extrinsic obstacles to cross-pollination.

Determining the nature of a species' breeding system is a critical step in designing rare plant management strategies (DeMauro, 1993; Hamrick et al. 1991). Too often, we attribute a species rarity to its inability to reproduce and this is rarely the case (Weller, 1994). As noted by Emery (1977, 1967), Terrell, et al. (1978), Emery and Guy (1979), and Power (1997), extrinsic factors contribute significantly to the reproductive failure of *Z. texana* in the wild. Habitat modification has resulted in fragmented, widely spaced stands. Floating mats of vegetation and debris, herbivory by introduced and nonmigratory waterfowl and nutria, competition from introduced plant species, and recreational use of the river reduce or eliminate the number of inflorescences available for pollen and pollination.

As Terrell (1978) noted, "the best means of preserving the species is by preserving the habitat intact and undisturbed." Barring this, a thorough understanding of the pollination biology and how it contributes to the reproductive success of *Z. texana* in the San Marcos River will not only guide *Z. texana* reintroduction and restoration efforts, but is critical for the conservation and effective management of the remaining population.

ACKNOWLEDGMENTS

The authors would like to thank Dr. David Bowles, Dr. Donald Koehler, Dr. Jim Ott, Dr. Damon Waitt, Dr. Mark Simmons, and Dr. Paula S. Williamson for their careful review of the project proposal; Dr. Carol Kearns, Dr. Norma Fowler, and Dr. Marcia Maues for their thoughtful advice and guidance in the development of the experimental design; Dr. Michael Longnecker and Lori Tolley-Jordan for their assistance with the statistical analysis; Leonard Oliver for his assistance with field work and data collection; and Dr. Tom Brandt for his support of the project.

Illustration by Claudia A. Harris.

CHAPTER 2

EFFECTS OF PANICLE DENSITY AND DISTANCE FROM POLLEN SOURCE ON POLLEN DISPERSAL AND SEED PRODUCTION

F. M. Oxley, Alison K. Echlin, and Paula Power

Abstract

A study was conducted in outdoor cement raceways at the San Marcos National Fish Hatchery and Technology Center examining pollen travel by *Zizania texana*. A split-plot design examined the effect of panicle density (four levels) and distance from a pollen source (six levels) on pollen dispersal. Pollen traps made from glass slides with petroleum jelly were placed at 0, 0.75, 1.5, 3.0, 6.0 and 12.0 m from panicles releasing pollen on four occasions: when panicle density was 4-8, 9-20, 21-40, and 41-100 panicles/1.6m². Pollen traps were in place for 24 hours. After 24 hours a cover slip was placed on each slide and the number of *Z. texana* pollen grains beneath the cover slip was counted and recorded. There were significantly more pollen grains on traps when panicle density was greater than 40 panicles/1.6m² ($p < 0.001$) compared with fewer panicles. There was significantly more pollen on traps within 0.75 m of panicles releasing pollen ($p < 0.001$).

Wind pollinated species commonly release pollen during specific intervals during a 24-hour cycle. *Zizania texana* pollen release was examined by collecting pollen at six-hour intervals for four consecutive 24-hour periods. In a second study, pollen was collected for one hour intervals for four 24 hour periods. In each study, the number of pollen grains on traps was counted and recorded. There was significantly more pollen on traps between 2300 hours and 0500 hours (nighttime; $p < 0.001$), compared with 0500 hours and 2300 hours (daytime). Slides placed hourly indicated peak pollen release was between 0200 hours and 0400 hours with a secondary release around 0900 hours.

INTRODUCTION

Flowering plants have evolved many pollination strategies, both biotic and abiotic, through time. Biotic pollination strategies are often complex and involve animal vectors to transport pollen from one flower to another (Northington and Goodin, 1984; Whitehead, 1969). Abiotic pollination involves either wind or water as the primary dispersal mechanism (Willson, 1983; Whitehead, 1969). Wind pollination, or anemophily, is believed to have evolved from biotic pollination strategies, primarily insect pollination (Whitehead, 1969). It has evolved independently several times in a number of different flowering plant families (Cook, 1988; Whitehead, 1969). Currently, it is recognized that wind pollination makes up 95-98 percent of all known examples of abiotic pollination and is the dominant mode of pollination in many plant families

(Proctor et al. 1996; Faegri and van der Pilj, 1971; Whitehead, 1969). Once considered inefficient, wind pollination may, in fact, be much more efficient than insect pollination (Proctor et al. 1996).

Wind pollinated species share a number of similar characteristics (Cook, 1988; Northington and Goodin, 1984). They are either dioecious or, if monoecious, pistillate and staminate flowers mature at different times (Proctor et al. 1996). They have well defined flowering seasons during which enormous amounts of pollen are produced and released (Proctor et al. 1996). Flower spikelets, composed of many small, inconspicuous flowers, are arranged in an inflorescence that is held above the foliage to facilitate access to wind currents (Proctor et al. 1996; Cook, 1988). Flowers have a reduced perianth, thus increasing their exposure to wind currents and pollen aerodynamic efficiency (Proctor et al. 1996; Cook, 1988; Whitehead, 1969). Stamens are large and positioned on long, flexible filaments to increase their exposure to air currents (Proctor et al. 1996; Cook, 1988; Northington and Goodin, 1984). Pollen grains are small, smooth, dry, and buoyant (Proctor et al. 1996; Willson, 1983). Like stamens, stigmas are also large and well exposed and specialized to increase the chances of capturing airborne pollen (Proctor et al. 1996; Cook, 1988; Crane, 1986; Whitehead, 1969). The number of ovules in wind pollinated flowers are reduced with only one seed per flower being produced (Proctor et al. 1996; Cook, 1988; Whitehead, 1969).

A number of factors affect successful dispersal of wind borne pollen. Temperature, humidity, wind currents, wind direction, distance pollen must travel, and spacing of plants all play a role in determining whether or not pollen reaches a receptive stigma (Faegri and van der Pilj, 1971). Anthers generally do not open unless the weather is warm and dry (Faegri and van der Pilj, 1971). Rainy weather quickly washes pollen out of the air (Faegri and van der Pilj, 1971). Individuals of wind pollinated species grow relatively close together to achieve successful pollination (Willson, 1983; Whitehead, 1969). Additionally, wind pollinated species grow in plant communities that have open structure or are deciduous at least part of the year to ensure that there are few obstructions present to inhibit pollen movement during their flowering season (Whitehead, 1969).

Zizania texana (Texas wild-rice), endemic to the San Marcos River, is a monoecious, aquatic macrophyte (Emery and Guy, 1979). *Zizania texana* grows in fragmented, widely dispersed stands throughout its limited range (Poole, 2002). Reproduction in the wild, until quite recently, has been primarily through vegetatively produced tillers. A wind pollinated species, it is believed that pollination may be a major impediment to successful sexual reproduction in the wild.

The objectives of this study were to determine: 1) how far *Z. texana* pollen can travel from source stands; 2) what effect panicle density and distance has on seed set; and, 3) the time of peak pollen release.

METHODS AND MATERIALS

All studies took place in four outdoor concrete raceways (17 m x 1.5 m x 0.9 m) at the National Fish Hatchery and Technology Center (NFHTC) located in San Marcos, Texas (Figure 2-1).



Figure 2-1. Concrete study raceways located at the National Fish Hatchery and Technology Center.

Each raceway was divided in half lengthwise using either a series of 2.4 m x 0.6 m sheets of 6 mm thick Plexiglas or a cinderblock divider. Seven submersible pumps were placed in each raceway to circulate the water and create a flowing water environment. Twenty-two pots, each containing one seedling, were placed at the drain end of each raceway in an area 1 m x 1.8 m to create four adjacent “stands” of Texas wild-rice.

The first study examined the effect of panicle density and the distance pollen travels from a pollen source on pollen distribution. Pollen traps were mounted on 1 m² frames constructed from 2.3 cm polyvinyl chloride (PVC) pipe. One frame was placed across each raceway at 0, 0.75, 1.5, 3, 6, and 12 m from the Texas wild rice 1.6 m² “stand” for a total of six collection frames per replicate raceway (Figure 2-1).

Pollen traps were constructed using glass slides coated with a thin layer of petroleum jelly. This medium was chosen because it is viscous, relatively unaffected by excess moisture, and remained sticky throughout the 24-hour collection period (Weis and Hermanutz, 1993).



Figure 2-2. Pollen traps were secured to the collection frames and remained in place for 24 hours.

At each distance interval, a pollen trap was secured to each PVC frame with ponytail fasteners (Figure 2-2).

Pollen traps remained in place for 24 hours. After 24 hours, the pollen traps were removed and returned to the lab. A cover slip was placed in the center of each slide (484 mm²) and pollen grains beneath the cover slip were counted and recorded. The presence of pollen grains on slides was considered a reliable indicator of the distance pollen traveled. Pollen traps were set out at each distance interval when the number of panicles releasing pollen in each of the four 1.6 m² stands was: 1) 4-8 panicles; 2) 9-20 panicles; 3) 21-40 panicles; and 4) 41-100 panicles. Excess panicles in the stand were emasculated when the number of panicles was greater than the desired range for each treatment. Pollen was collected for three days for each panicle density treatment.

The design was a split-plot design because we were unable to completely randomize the order of runs. Panicles became increasingly more abundant as the season progressed; therefore, experimental runs were consecutive rather than concurrent. Panicle densities were whole plots. Each whole plot was divided into six split-plots and one distance interval was assigned to each split-plot. Data from three consecutive days were averaged for each treatment followed by log (x + 1) transformation before analysis (Montgomery, 2001; Zar, 1984). A Tukey-Kramer post hoc comparison test was used to determine significant differences between treatments.

A second study was undertaken to determine the effect of panicle density and distance from pollen source on seed set. Potted *Z. texana* seedlings were placed at each distance interval. For each panicle density (4-8, 9-20, 21-40, 41-100), one experimental panicle with receptive stigmas at each distance interval was selected and tagged. Panicles were tagged for three consecutive days. When tagged panicles matured to the staminate stage, they were emasculated and each panicle bagged with a fabric bag. After 10 days, bagged

panicles were clipped and returned to the lab. The presence or absence of maturing seeds on each panicle was recorded.

To determine at what time pollen is released during a 24-hour period, pollen was collected at six-hour intervals (0 – 0500 hours; 0600 – 1100 hours; 1200 – 1700 hours; 1800 – 2300 hours) for four consecutive 24-hour time periods. Pollen traps were set out when stands had at least 20 panicles releasing pollen. Two pollen traps were placed within each “stand” in each raceway for a total of eight traps per time interval. At the end of each time interval, traps were removed and returned to the lab. Cover slips were placed in the middle of each slide and pollen grains counted and recorded. Data were transformed using the $\log(x + 1)$ transformation and analyzed using a one-way ANOVA with repeated measures. The Tukey-Kramer post hoc comparison test was used to determine significant differences between time intervals ($\alpha = 0.05$). This question was further examined by placing two pollen traps in the “stand” for each hour for 24 hours. At the end of each hour, slides were removed and returned to the lab. Cover slips were placed in the middle of each slide and pollen grains counted and recorded. This was repeated for four consecutive days.

During the entire study, a weather station (CR10X, Campbell Scientific, Inc.) located within 300 feet of the experimental site recorded the following weather data: temperature, precipitation, relative humidity, barometric pressure, solar radiation, wind speed, and wind direction.

RESULTS

The number of panicles releasing pollen and the distance from the pollen source significantly affected the number of pollen grains on slides. The number of grains on slides was significantly greater when the number of panicles/ 1.6 m^2 was 40 or more ($p < 0.001$). No difference in the amount of pollen deposited was observed when panicle density was less than 40/ 1.6 m^2 (Figure 2-3; Table 2-1).

There was significantly more pollen deposited on slides within 0.75 m of the pollen source compared with all other distance intervals ($p < 0.001$) and there was progressively less pollen on slides as distance increased. Slides collected 12 m from the pollen source had significantly less pollen than at 0, 0.75, 1.5, and 3 m intervals (Figure 2-5). There was no interaction between factors ($p = 0.368$).

There were too few study plants with receptive stigmas present during the study period to statistically analyze the effect of panicle density and distance interval on seed set. Seed set data is summarized in Appendix 2-2.

Table 2-1. Mean number of *Zizania texana* pollen grains per 484 mm². N=4; standard error in parentheses. There was significantly more pollen on slides when panicle density was greater than 40/1.6 m and distance from pollen source was less than 0.75 m.

Distance (m)	Panicle Density/1.6 m ²			
	4-8	9-20	21-40	41-100
0	12.6 (5.4)	46.9 (19.9)	69.7 (36.8)	314.3 (67.2)
0.75	7.2 (4.2)	36.3 (16.7)	16.8 (9.9)	103.1 (16.7)
1.5	6.4 (2.8)	17.8 (6.7)	15.3 (9.1)	60.7 (11.6)
3	7.1 (3.4)	8.8 (3.1)	5.2 (2.4)	21.4 (5.1)
6	6.1 (3.2)	3.7 (0.6)	1.9 (0.6)	9.6 (1.9)
12	4.7 (2.1)	2.3 (0.6)	1.7 (0.6)	6.7 (1.3)

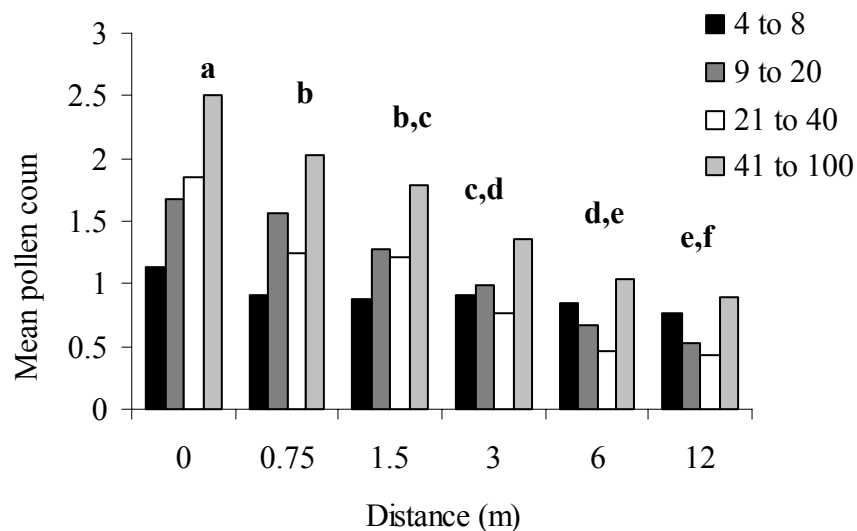


Figure 2-3. Mean number of pollen grains (log x + 1) on pollen traps as a function of panicle density and distance traveled. There was significantly more pollen on traps within 0.75 m of the pollen source and when the pollen source had greater than 40 panicles releasing pollen. Panicle density is represented by colored bars. Identical letters above bars indicate groups of means with no statistically significant differences at the 95% confidence level.

Zizania texana primarily releases pollen during the night. The number of pollen grains counted on slides was significantly greater between 2300 hours and 0500 hours (nighttime) ($p < 0.001$), compared with 0500 and 2300 hours (daytime). Slides placed hourly at the study site indicated peak pollen release was between 0200 hours and 0400 hours with a secondary release around 0900 hours (Figure 2-4).

Weather conditions during the hourly pollen dispersal study periods are summarized in Table 2-2. Weather conditions throughout the study period are summarized in Figure 2-6.

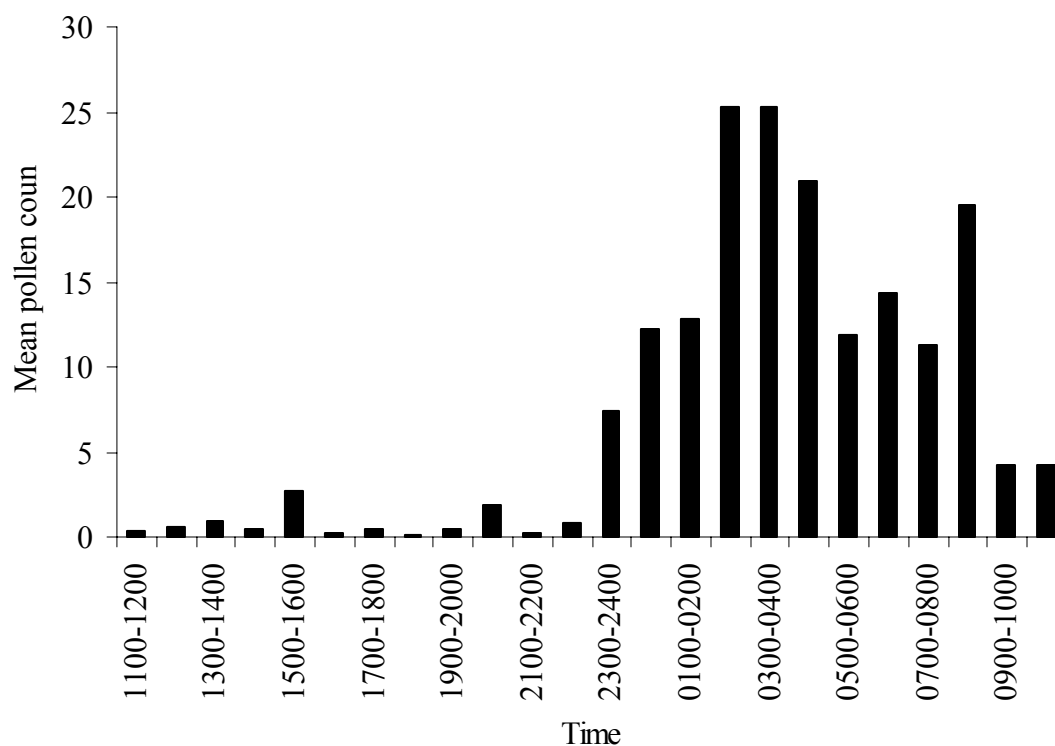


Figure 2-4. Mean number of *Zizania texana* pollen grains on pollen traps. Pollen was released throughout the 24-hour cycle (n = 4) with two peak releases, one between 0200 and 0400 hours and second, smaller peak between 0800 and 0900 hours.

Table 2-2. Weather conditions during the two hourly pollen dispersal studies, June 24 – 27, 2003 and July 21 – 24, 2003.

Time	Ave Air Temp °C	Ave RH %	Ave kW m ⁻²	Ave WS m/s	Ave Rainfall mm/day	Ave Pressure mb
0-0500 hours	23.75	88.46	0.00	1.48	0.39	991.66
0600-1100 hours	24.54	85.77	0.19	1.89	0.49	992.50
1200-1700 hours	30.37	59.25	0.65	2.86	0.22	991.54
1800-2300 hours	28.19	67.55	0.12	2.36	0.14	990.51

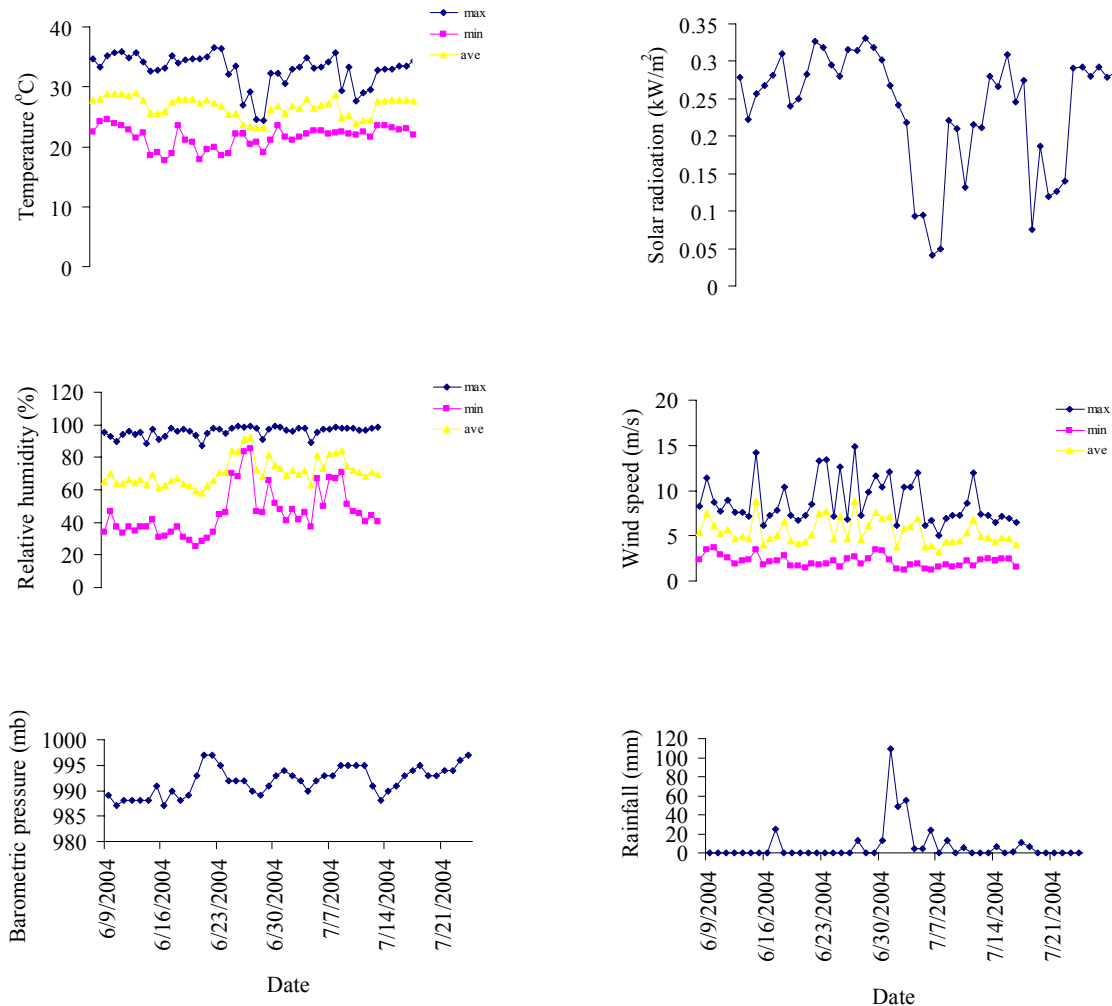


Figure 2-5. Weather conditions throughout the study period, June 9 – July 24, 2003.

DISCUSSION

In most wind pollinated species, pollen is released throughout the day and many species exhibit some degree of periodicity of pollen release (Bianchi et al., 1959; Jones and Brown, 1951; Meinders and Jones, 1950). Typically, pollen is released early in the day, usually before noon and may include one or more peaks (Beddows, 1959; Jones and Brown, 1951; Meinders and Jones, 1950; Wolf, 1925). *Zizania texana* exhibits the same general trend throughout the day; however, unlike many other wind pollinated species, *Z. texana* releases most of its pollen during the pre-dawn hours, between 0200 and 0400 hours with a second, minor release period between 0800 and 0900 hours.

In general, solar radiation, low humidity, and moderate temperatures favor pollen release (Potter and Rowley, 1960; Meinders and Jones, 1950). However, humidity plays the primary role in anther dehiscence and, ultimately, pollen release (Hodgson, 1949). As humidity decreases, anthers dehydrate, split open, and expose pollen (Schmid, 1976; Faegri and van der Pijl, 1971; Payne, 1963; Burton, 1942). Wind currents and gravity

then disperse the pollen grains. For example, in studies of the common ragweed (*Ambrosia artemisiifolia*), Bianchi, et al. (1959) found that as temperatures rose and relative humidity decreased, anther dehiscence and pollen release occurred. Similarly, Meinders and Jones (1950) observed the same trend in the castor plant (*Ricinus communis*).

In direct contrast to what has been observed in other wind pollinated species, *Z. texana* releases pollen during periods when the humidity is high and temperatures are low relative to other times during the 24-hour cycle. Relative humidity and temperature during the two peak pollen release periods averaged 89.9% RH and 23.5°C (0200-0400 h) and 88.6% RH and 24.1°C (0800-0900 h). Relative humidity and temperature during the period of lowest pollen release (1100-2300 h) averaged 63.2% RH and 29.3°C (Table 2-2).

While wind dispersed pollen can be carried long distances, most is deposited very close to the parent source (Proctor et al. 1996; Willson, 1983). As distance increases from the parent source, the pollen load in the air quickly decreases (Wright, 1953; Haskell and Dow, 1951; Meinders and Jones, 1950; Hodgson, 1949). Even if pollen grains do travel several kilometers from the source, they are rarely viable once they fall out of the air (Willson, 1983).

The pollen of *Z. texana* did not travel far from its source in this study, even when there were more than 40 panicles/1.6 m² releasing pollen. The majority of *Z. texana* pollen was collected at the 0, 0.75, and 1.5 m intervals and few, if any, pollen grains were collected at distance intervals greater than 1.5 m. This may be a function of pollen grain size, weight, and buoyancy. Smaller grains are more buoyant than larger grains while larger, heavier pollen grains tend to fall out of the air faster and travel shorter distances (Faegri and van der Pilj, 1971). Preliminary estimates of *Z. texana* pollen grain size (~35µm diameter) indicate that it is similar in size to that of *Z. palustris* (35 – 40 µm) (*pers. comm.*, Raymie Porter, University of Minnesota) and further study is needed to determine if grain size is an important factor in pollen flow within and among stands.

Wind pollinated species typically grow in large groups or colonies and are termed “gregarious,” a condition considered necessary for successful wind pollination (Faegri and van der Pilj, 1971). Because they rely on abundant pollen and wind currents to transport it to compatible flowers, wind pollinated species growing in fragmented or small, isolated populations are at a disadvantage when it comes to achieving successful pollination (Faegri and van der Pilj, 1971).

The distance pollen travels becomes important when considered from a genetic point of view (Wright, 1953). Species with limited pollen dispersal distance tend to have frequent unbridged gaps in their range, gene flow between stands is limited, heterogeneity is reduced, and distinct local and geographic races may result (Potter and Rowley, 1960). In contrast, in species capable of large pollen dispersal distances, we would expect to see few gaps in range, increased gene flow between populations, and no distinct local and geographic races (Potter and Rowley, 1960).

Three lines of evidence suggest that *Z. texana* is a gregarious species. First, in this study most pollen traveled less than 1.5 m and lived less than 10 minutes after release from the anthers. For successful pollination to occur, *Z. texana* must grow in large colonies, with many male and female flowers in different developmental stages blooming simultaneously. Under artificial conditions in the raceways, significantly more pollen grains were found on pollen traps when 40-100 inflorescences were blooming compared with 40 or fewer blooming inflorescences.

Second, annual monitoring of *Z. texana* by Texas Parks and Wildlife Department during the past 10 years has shown an increase in aerial cover in a 100 m section of river downstream from University Drive (Poole, 2002). At the present time, a nearly continuous stand with emergent culms scattered throughout in spring and summer and extending approximately 60 m long and 10 m wide occurs in the area. In 1998, for the first time in at least 30 years (Emery, 1967), seeds were produced by *Z. texana* in this large stand, while seed production was all but absent in every other part of its range where stands are smaller and widely spaced.

Third, a genetic study of *Z. texana* was recently completed by Chris Richards of the USDA's National Center for Genetic Resources Preservation. Richards (*pers. comm.*) found that *Z. texana* has a high degree of genetic heterogeneity and that there is no geographic segregation of alleles among or within wild stands. This suggests that, at some point in time, there was gene flow throughout the population and groups or individuals were not isolated from each other.

The patchy distribution characteristic of today's wild population is a modern phenomenon, and an artifact of human modification of the habitat. Studies by Saunders et al. (2001) indicate that the preferred habitat for *Z. texana* is water depth of 0.23 – 0.91 m combined with current velocity of 0.06 – 0.61 m/s and coarse sandy soils. The construction of four dams along the San Marcos River in the last 150 years has impacted designated critical habitat for *Z. texana*. The dams divide the habitat into a series of alternating upstream impoundments and downstream runs and riffles. Impoundments eliminate large areas of preferred habitat for *Z. texana*, and result in habitat with water depths greater than one meter, nearly undetectable current velocity, and sediments of sand, silt, and clay (Saunders et al. 2001). Such habitat is not capable of supporting healthy stands of *Z. texana*. Dams effectively divide the *Z. texana* population into three geographically separate locations all downstream from dams where runs are present.

The impoundments also impede the dispersal of reproductive propagules, both seeds and vegetatively produced tillers. Any seed or asexual clone produced will likely sink to the bottom before it can float through an impoundment and over a dam to preferred habitat. In downstream habitat, there are fewer new recruits; individuals senesce and stands have become progressively smaller. What was once large, gregarious stands, now are relict, isolated patches.

A number of disturbances, human caused or natural, create opportunities for colonization by more aggressive species. *Hydrilla verticellata* (Hydrilla), *Hygrophila polysperma*

(*Hygrophila*), and *Cryptocoryne beckettii* (Water trumpet) are but a few of the species that take advantage of these opportunities, precluding recruitment and colonization by *Z. texana* in suitable microhabitats.

Sand, gravel, and debris are deposited at the tributaries of all major creeks. Often a consequence of poor management in the watershed during construction, road maintenance, or overgrazing, the deposited material is unstable, buries existing vegetation, changes the nature of historic sediment type, and alters existing water depth and flow patterns. Once again, this type of disturbance favors more aggressive colonizers. Sediment erosion can also destabilize *Z. texana*. Eroding sediments expose the root zone and often a part of, or, the entire stand washes away.

Unfortunately, even in well developed, large stands, other disturbances can impede pollination. Grazing of culms, submersion of emergent culms by waterfowl and floating vegetation mats, breaking or shredding of culms, and interference with culm emergence all slow or even prevent flowers from blooming (Power, 1996b; Power, 1997).

The outcome of these types of disturbances is patchy distribution and small stands, leading, ultimately, to reproductive failure at the pollination stage. The distance between stands becomes too great for effective pollen dispersal and stands smaller than about 3 m² produce neither enough emergent blooming culms necessary for the amount of pollen required for successful pollination to occur, nor enough receptive stigmas to receive it. Without successful pollination, there is no seed set and ultimately no recruitment of new seedlings from seed. Stands become progressively smaller, further reducing the chances of successful pollination, and placing each stand in jeopardy of local extinction.

ACKNOWLEDGMENTS

The authors would like to thank Dr. David Bowles for his careful review of the project proposal; Dr. Michael Longnecker for his assistance with the statistical analysis; Lori Tolley-Jordan for her assistance with fieldwork and data collection; and Dr. Tom Brandt for his support of the project.

CHAPTER 3

SEASONAL VARIATION IN SEED PRODUCTION

Paula Power

INTRODUCTION

In 1986, Paul Fonteyn, former professor from Texas State University (TSU) and the author collected seven clumps of *Zizania texana* from the IH35 area of the San Marcos River. Plants were brought to the TSU campus for the purpose of establishing a seed-producing population. Over time, additional wild plants were brought to TSU, together with excess tillers from the refugium population at the National Fish Hatchery and Technology Center (NFHTC). Seeds were collected each year. Seeds were ultimately used for research and reintroduction projects, and to replenish the seed-producing population. For a history of the captive population at TSU from 1986 to 1996 see Rose and Power (2001).

METHODS AND MATERIALS

In 1999, a seed-producing population was established at NFHTC. During the years 1999 to 2003, seeds were collected from both the TSU and NFHTC population. Seeds were weighed and during some years, counted and divided into seeds that sink or float when dropped in water. The division of seeds into these two groups simulates a situation in which seeds drop from emergent culms and either sink through the water column to the river bottom or float downstream. Often seeds that float eventually sink, but the time before sinking varies from minutes to hours. Following collection, seeds were placed in moist paper towels in Ziplock[®] style plastic bags and stored in a refrigerator. The collection data provide insight into seasonal variation in seed quality and production. This chapter is a compilation of seed collection data between 1999 and 2003.

RESULTS

Data from 1999, 2000, 2001, 2002, and 2003 were used to calculate monthly mean seed production. Seeds were not produced every month in every year, but, on average, seeds were produced every month of the year with peak production during March, April, May, and June (Table 3-1). The month with the greatest mean seed production was June with 85.69 g (± 132.03) (Figure 3-1).

Table 3-1. Total monthly seed harvest from the *Zizania texana* seed producing population at the National Fish Hatchery and Technology Center and the now defunct Texas State University population.

Year	Month											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1999			83.43	204.29	134.08	87.93	9.45	5.17	1.71	3.77	7.83	1.54
2000	0.5	0.5		0.69	15.53	17.20	18.15	3.79	2.50	19.8	24.31	14.51
2001	5.76	1.12	0.66	23.07	77.85	313.62	44.08	5.62	1.24	15.27	5.03	
2002						3.602	15.666	17.018	14.398	24.970	11.390	
2003					0.51	6.105	10.155	4.312	5.88	0.18		
Mean	3.13	0.58	42.04	76.02	56.99	85.69	19.50	7.18	5.14	12.80	12.14	8.02
STDEV	3.72	0.77	58.53	111.65	61.34	132.02	14.22	5.54	5.48	10.53	8.52	9.17

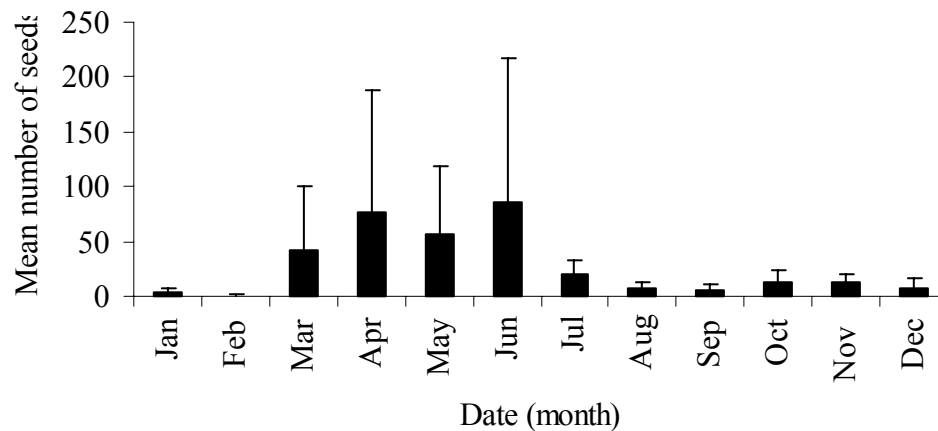


Figure 3-1. Mean number of *Zizania texana* seeds produced during years 1999-2003.

During 1999 and 2000, the number of inflorescences from which seeds were collected was recorded. The mean number of seeds produced per inflorescence was 16.6 for 1999 and 2000 combined. There was seasonal variation in the number of inflorescences and the number of seeds per inflorescences. The month with greatest number of inflorescences producing seed was June followed by April and May. The greatest mean number of seeds per inflorescences was April with 26.2 seeds per inflorescence (± 3.1) followed by March, May and June with 19.0, 12.7, and 13.1, respectively (Table 3-2).

There was variation in the weight of seeds produced both seasonally and when comparing seeds that sink or float. Of seeds that sink, those with the greatest mean weight (0.015 g per seed) were produced in March, April, May, and December (Table 3-3). Those with the least mean weight (0.011 g per seed) were produced during August and January. Of seeds that float when dropped in water, those with the greatest mean weight (0.012 g per seed) were produced during May. Those with the least mean weight (0.008 g per seed) were produced during January, March, August, and December.

Table 3-2. Mean number of *Zizania texana* seeds, mean number of inflorescences, and mean number of seeds per inflorescence produced during the months of March through November during 1999 and 2000.

	Month								
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Number of seeds	2,443	15,050	9,160	8637	2142	581	202	242	86
Number of inflorescences	114	586	577	706	196	82	27	21	10
Mean number of seeds per inflorescence	19.0	26.2	12.7	13.1	10.3	9.9	7.0	12.4	8.6
SDev	9.4	3.1	6.1	2.5	2.4	4.5	5.3	7.6	-

Table 3-3. Mean weight of *Zizania texana* seeds that sink or float when dropped in water. Weights are from seeds collected during the months of January through December during 1999, 2000, 2001, 2002, 2003. Seeds were collected from the *Z. texana* seed producing population at the San Marcos National Fish Hatchery and Technology Center and the now defunct Texas State University population.

Month	Sink		Float	
	Weight (g)	Proportion of total	Weight	Proportion of total
January	0.011	0.09	0.008	0.91
February	-	0.0	0.009	1.0
March	0.015	0.11	0.008	0.89
April	0.015	0.79	0.011	0.21
May	0.015	0.77	0.012	0.23
June	0.013	0.47	0.011	0.53
July	0.013	0.32	0.010	0.68
August	0.011	0.28	0.008	0.73
September	0.012	0.24	0.010	0.76
October	0.013	0.43	0.010	0.57
November	0.014	0.38	0.011	0.62
December	0.015	0.34	0.008	0.66

There was also seasonal variation in the proportions of seeds that either sink or float. During April and May, 79% and 77%, respectively, of all seeds produced sank when dropped in water. During the rest of the year, more than half of all seeds produced floated when dropped in water.

DISCUSSION

Compared with many species of flowering plants, *Z. texana* has a long bloom period. While most species produce flowers for a matter of weeks, *Z. texana* is capable of producing flowers every month of the year. The peak reproductive period is April, May, and June. More inflorescences are produced during this time than any other time of year, each releasing pollen to pollinate and ultimately fertilize pistillate flowers. A regression analysis of the data showed a slight positive relationship between the number of inflorescences and the number of seed per inflorescence ($p < 0.05$; $y = 9.906 + (0.058)x$; $R^2 = 0.289$). This supports the results of Chapter 2, which found significantly more pollen available to pollinate and fertilize pistillate flowers when there were more than 40 inflorescences/1.6 m² compared with fewer inflorescences.

Seeds used in this assessment were divided into two categories, seeds that sink or seeds that float when dropped in water. Seeds that sink are significantly heavier and have significantly higher percent germination compared with seeds that float (74% vs. 48% germination). The two categories were chosen to simulate natural conditions. A seed falls from an emergent culm when mature and drops through the water column to the river bottom. The current may carry the seed away from the parent plant and the seed may roll along the bottom until it lodges behind or beneath gravel or rocks (see Power and Fonteyn, 1995 for a discussion of germination requirements and seedling vigor). There it may germinate and grow into a new plant. The fate of seeds that float on the water surface is less clear. Seeds that float can be carried much greater distances from the parent plant, and may eventually sink.

The physiological explanation for the differences between seed categories is unclear. It may be the result of different developmental pathways (Vertucci et al. 1995; 1994). This requires further study.

The proportion and weight of sinkers is greatest during the most productive months, March, April, May, and June. The months with the highest proportion of seeds that sink are April and May (79% and 77%, respectively) and the months with the heaviest seed are March, April, and May (0.015 g per seed). March through June is the time of the year when the most robust seed with the highest percent germination are produced (Chapter 4). If the management objective is to promote seed production, March through June is the time of year to protect *Z. texana* from disturbance, which interferes with reproduction.

ACKNOWLEDGMENTS

Special thanks to Tyson Galusky, Michele Guardiola, and Virginia Rodriguez for their dedication and commitment to harvesting and processing *Zizania texana* seeds.

CHAPTER 4

SEED QUALITY

Alison K. Echlin, F.M. Oxley, and Paula Power

Abstract

Seed weight, seed germination, and seedling development of seeds that sink and seeds that float were studied in seeds of *Zizania texana*. Seeds were cold stratified at 3°C for 16 weeks. Five hundred sinkers and 500 floaters were germinated in glass vials in an environmental chamber and the germinated seeds were allowed to develop in 125 ml flasks in an environmental chamber set at 21°C and 12 hours light/12 hours dark. Seeds that sink in water are significantly heavier than seeds that float. Seeds that sink have higher percent germination than seeds that float. There was no difference in seedling development between seeds that sink and seeds that float.

INTRODUCTION

New plant life begins with the germination of a seed (Northington and Goodin, 1984). Defined as the resumption of growth and an increase of metabolic activity, germination is a complex physiological process that can be affected by a number of factors including climate, chemical inhibitors, seed age, and thickness of seed coats (Seed Biology Place, 2003; Texas A&M University, 2003; Baskin and Baskin, 1998). A seed will not normally germinate unless environmental factors are favorable (Stern, 1979). For example, the seeds of some species require low oxygen levels in order to germinate (Power and Fonteyn, 1995), while others require specific temperature regimes (Whigham and Simpson, 1982).

A mature seed contains a complete embryo. As it germinates, the embryo simply begins expanding existing tissues and adding new ones (Northington and Goodin, 1984). While seed size within a plant species has traditionally been considered to be a constant characteristic (Hendrix, 1984), many studies have shown that it can vary greatly within species and even among seeds produced by an individual plant (Thompson, 1984; Gross and Soule, 1981; Schaal, 1980). It would be expected that, when other factors remain constant, seed size would play a primary role in determining which individuals would succeed within a population (Stanton, 1984).

A number of studies have shown that seed size can influence whether or not a seed will germinate (Roach, 1987; Gross, 1984; Schaal, 1980). Large seeds often have higher germination rates than smaller seeds (Gross 1984; Hendrix, 1984). Smaller seeds may germinate more quickly providing their offspring with a competitive advantage (Hendrix, 1984; Grime et al. 1981; Howell, 1981; Ross and Harper, 1972) over larger seeds.

Seedling development, like seed germination, depends on a number of environmental factors. These factors include temperature, oxygen, and moisture levels (Northington and Goodin, 1984). Some species possess seedling vigor and are adapted to withstand environmental stresses, thus increasing their chances of survival (Northington and Goodin, 1984). Because bigger seeds have larger food reserves, they produce larger and more vigorous seedlings (Dolan, 1984; Gross and Soule, 1981; Schaal, 1980; Baker, 1972). Small seeds have lower germination rates and produce much smaller seedlings with lower survivorship; however, the sheer numbers of small seeds produced ensure reproductive success (Schaal, 1980; Baker, 1972;).

Zizania texana (Texas wild rice) is a monoecious, aquatic perennial endemic to the San Marcos River. Blooming March through November, *Z. texana* produces seeds that, when released from the panicle, either sink or float. The purpose of this study is to examine the differences in seed weight, seed germination, and seedling development between seeds that sink and seeds that float.

METHODS AND MATERIALS

All seeds used in the studies were collected from *Zizania texana* plants grown at the San Marcos National Fish Hatchery and Technology Center, San Marcos, Texas. After collection, seeds were dropped into a pail of water and separated into two groups: seeds that sink and seeds that float. Seeds from each group were then placed on moist paper towels, sealed in Ziploc® type storage bags, and placed in a refrigerator set at 3°C.

After 16 weeks of cold stratification, mean weight of seeds that sink and seeds that float was examined. Fifty sinkers and 50 floaters (collected on 3 July 2003) were removed from storage, the lemma and palea removed, and weighed in a tared aluminum weigh boat using a Mettler-Toledo AB204-S balance. The weight of each seed was recorded. Weighed seeds were then placed back into Ziploc® type storage bags and stored in the refrigerator at 3°C. Data were analyzed using a two-tailed *t*-test.

Percent germination was examined using seeds from three collection dates: 3 July, 7 July and 11 July 2003. There were 50 replicates for each treatment. For each replicate, 10 seeds were placed in a vial filled with tap water and capped. Vials were labeled with treatment and date and placed on a rack in an environmental chamber set at 12 hours light/12 hours dark and 21°C. After a 10-day incubation period, the number of germinated seeds from each vial was counted and recorded. Seeds were considered germinated if the shoot had emerged and was at least half as long as the seed. Data were converted to proportions (number seeds germinated/total number seeds in vial), transformed using arcsine transformation, and analyzed using a two-tailed *t*-test.

Differences in seedling development between sinkers and floaters were determined using germinated seeds from the previous study. Seedlings were placed in 125 ml flasks filled with 150 ml of tap water. There were 12 flasks containing sinkers and 10 flasks containing floaters. Flasks were placed in an environmental chamber set at 12 hours light/12 hours dark and 21°C. After a 10-day incubation period, seedlings were sorted

and counted using the following categories: 1) no chlorophyll, no roots; 2) chlorophyll present, roots absent; 3) chlorophyll present, roots present. Data were recorded and analyzed using a chi-square 2 x 3 contingency table.

RESULTS

The Kolmogorov-Smirnov Normality test was used to determine if seed weight data were normally distributed. Although ‘floater’ data distribution departed from normality, the two-tailed t-test was considered robust enough due to equality of variances and equal sample sizes (Zar, 1999). Results for seed weight showed that seeds that sink in water were significantly heavier than seeds that float in water ($t_{98, 0.05} = 9.38$, $p < 0.0001$). Mean weight of sinkers was 0.0097g and mean weight of floaters was 0.0066 g (Figure 4-1).

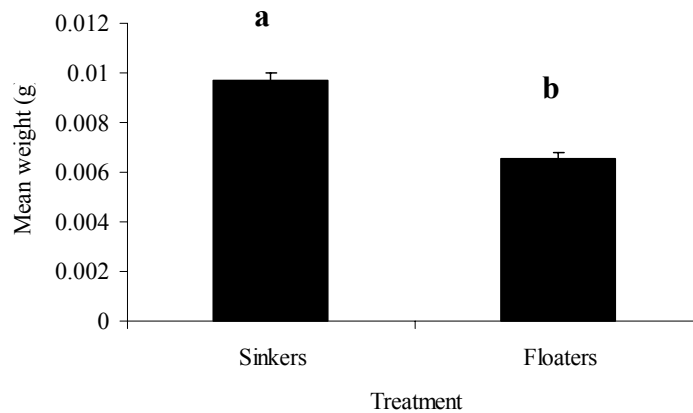


Figure 4-1. Mean weight of sinkers and floaters ($0.0097\text{g} \pm 0.0003\text{g}$ and $0.0066\text{g} \pm 0.0002\text{g}$, respectively). Mean weight of sinkers was significantly greater than mean weight of floaters ($p < 0.001$).

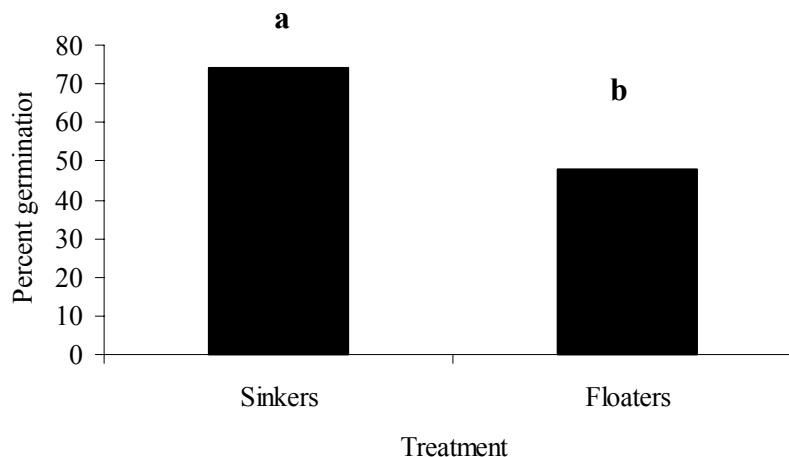


Figure 4-2. Percent germination of sinkers and floaters (74% vs. 48%). Sinkers had significantly greater percent germination than floaters ($p = 0.0001$).

Results of the two-tailed t -test for percent germination showed that seeds that sink have a significantly higher percent germination than seeds that float ($t_{98, 0.05}=8.18$, $p=0.0001$). Seeds that sink ($n=50$) had a 74% germination rate, while seeds that float ($n=50$) had only a 48% germination rate (Figure 4-2).

There were no significant differences in seedling development between sinkers and floaters ($\chi^2_2 = 2.06$, $1.386 < p < 2.773$). Of 371 sinkers, 23% had no development (no chlorophyll and no roots, although shoots did grow somewhat), 20% had intermediate development (chlorophyll and no roots), and 57% exhibited full development (chlorophyll and roots present). Of 238 floaters, 26% had no development (no chlorophyll and no roots), 22% had intermediate development (chlorophyll and no roots), and 51% exhibited full development (chlorophyll and roots present) (Figure 4-3).

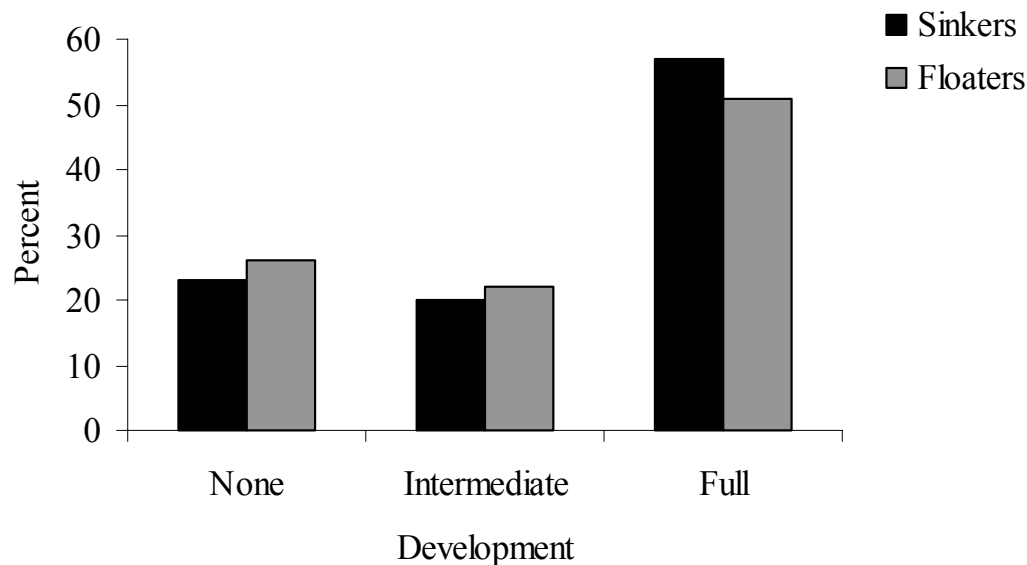


Figure 4-3. Percent of seedlings exhibiting no development (no chlorophyll, no roots), intermediate development (chlorophyll, no roots), and full development (chlorophyll, roots).

DISCUSSION

There has been some recruitment by *Zizania texana* in the wild but it is assumed to be by asexual tillers rather than by seed (*pers. comm.*, Jackie Poole, Texas parks and Wildlife Department). Prior to 1998, no seed production had been observed in the wild for more than 30 years. Environmental conditions in one small area of the range (Sewell Park) changed leading to seed production each year since 1998. Three major floods occurred, in 1998, 2001, and 2002, reducing the likelihood and our ability to observe and document recruitment by seed in Sewell Park.

Mature seeds that drop from panicles follow one of two pathways: they either immediately sink to the river bottom near the parent plant or float on the surface for sometime, drifting with the current away from the parent plant.

In *Z. texana*, sinkers have higher percent germination, which may give them an adaptive advantage in nature. Seedlings arising from heavier seeds have a better chance of becoming established in competitive or stressful habitats because they contain larger food reserves (Baker, 1972). There are, however, limitations and tradeoffs for larger seeds, including limited available resources, reduced space in fruit, less dispersal ability, and predator preference (Dolan, 1984; Schaal, 1980; Baker, 1972).

Seeds that float, on the other hand, have lower percent germination than sinkers. In order for species that produce smaller seeds to be successful in competitive habitats, they need to produce greater numbers of seeds (Baker, 1972). Greater numbers allow these species an opportunity for at least one seed to reach a favorable spot and germinate (Baker, 1972). Smaller seeds are more easily dispersed, allowing them to reach and potentially colonize new habitats (Baker, 1972). As with larger seeds, there are limitations and tradeoffs associated with being smaller. Disadvantages of smaller seed size include less food reserves leading to reduced seedling survivorship and competitive ability (Harper, 1977).

Regardless of whether a seed sinks or floats, there is no difference in seedling development. Each type of seed has its own advantage. Sinkers germinate where they fall and have larger energy reserves, which give seedlings a better chance of survivorship in competitive or stressful environments (Baker, 1972). Floaters are more easily dispersed and able to float away from the parent population, providing an opportunity to colonize new habitats (Baker, 1972).

The lack of recruitment in the wild population may be explained by the effects of floods, decomposers, and herbivores, or sinkers may germinate and produce seedlings are out competed by larger, established plants. Floaters may simply be floating out of range and failing to settle in appropriate habitat and establish new colonies. Further study of seed dispersal ability and distances seeds travel is needed.

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CHAPTER 5

FIELD SURVEY OF REPRODUCTIVE ACTIVITY OF *ZIZANIA TEXANA* IN THE SAN MARCOS RIVER WITH SUPPORTING EVIDENCE FROM A GREENHOUSE STUDY

Paula Power, Lori Tolley-Jordan, and F.M. Oxley

Abstract

A survey of the wild *Zizania texana* population was conducted to quantify reproductive activity, including estimating culm density and the number of culms exhibiting an impediment to reproduction. A two-stage random sampling method was used to sample *Z. texana*. Forty-eight 30.5 m segments, which contained *Z. texana* were identified. Within each segment, transects were placed randomly across the river at 3.10 m intervals, then 0.065 m² quadrats were placed randomly along each transect at 3.05 m intervals. Water depth, current velocity, culm density and culm condition were recorded for each quadrat. The mean number of culms per quadrat was 8.03. One percent of culms were in the seed-producing stage. Forty-five percent were either submersed and developing or emergent and were potentially reproductive. Fifty-four percent of the culms were either broken, shredded, or were submerged during floral development. These data support the prevailing opinion that extrinsic factors are responsible for reproductive failure in *Z. texana*.

INTRODUCTION

Reproductive failure in *Zizania texana* has long been identified as a threat to the species (Emery, 1967; 1977; U.S. Fish and Wildlife Service, 1996). Emery and Guy (1979) and Power (1997) both reported that *Z. texana* produces viable offspring and that impediments to sexual reproduction are due to extrinsic factors in the habitat. Previous studies on *Z. texana* indicated the species produces reproductive culms when grown in designated critical habitat and that herbivory of emergent culms and floating vegetation mats may play a role in reproductive failure (Power, 1996a; 1996b; 1996c). Prior to 1998, emergent reproductive culms were rare in the wild; however, since 1998, emergent culms have become common during spring and summer in Sewell Park, while observations of seed-producing culms are still rare in the remaining population in other parts of the river. The purpose of this survey was to quantify reproductive activity, including culm density and the number of culms exhibiting an impediment to reproduction (e.g. broken culm or submerged inflorescence). Furthermore, because *Z. texana* is a clonal species growing in large stands with clones rooting very near the parent plant, it is difficult, if not impossible, to distinguish individuals in the field. And, while a survey of wild stands provides information about reproductive activity per unit area, it does not provide

information about the reproductive activity of individuals. Therefore, a greenhouse study was designed and implemented that examined the asexual reproductive activity of individual *Z. texana* plants.

METHODS AND MATERIALS

Designated critical habitat for *Z. texana* extends 8,138 m (26,700') from the headwaters of the San Marcos River in Spring Lake to the confluence of the San Marcos and Blanco rivers. The actual range of *Z. texana* extends 4,176 m (13,700') from the dam forming Spring Lake to the power line that crosses the river near the A.E. Wood State Fish Hatchery. Plants occur in clusters called "stands." Stands are highly variable in size and have a patchy distribution in the river. In this survey, we sampled reproductive culms within "stands" using two-stage sampling. The first stage located random stands and the second stage located random plants within a stand (Elzinga et al. 1998). The river was divided into 30.5 m segments from Spring Lake to the City of San Marcos waste water treatment plant. Each 30.5 m segment of the river was assigned a number. Using a random number table, segments were selected until 48 segments containing *Z. texana* were identified. These were the primary sampling units. Forty-eight segments were determined to be a sample size that would provide an accurate estimate of the population (Elzinga et al. 1998). Each 30.5 m segment was subsampled (secondary sampling unit) as follows: five transects were placed across the river at 6.1 m intervals. The starting point for placing the first transect was randomly selected. A number between 0 and 6.1 was randomly selected to represent the starting point for the first transect and the remaining transects were placed at 6.1 m intervals from the starting point. Along each transect a quadrat 0.25 m x 0.25 m was placed every 3.05 m. The start point for placing the first quadrat on each transect was random. All quadrats were 3.05 m apart. Only transects and quadrats intersecting *Z. texana* were used.

The following information was recorded for each quadrat: total number of culms; number of tillers; submersed, developing culms; broken culms; shredded culms; submerged, mature inflorescence; emergent culms; and seed-producing culms. The presence or absence of floating vegetation was recorded and, if present, percent cover of the vegetation was recorded. The species of plant neighboring the quadrat upstream, downstream, on river left and on river right were recorded. Finally, current velocity and water depth were measured at the upstream side of each quadrat and recorded.

The mean and standard deviation for each parameter with quantitative data were calculated and are reported here. Qualitative data were summarized. Quadrats were divided into two groups, those with and those without floating mats. Data from the two groups of quadrats was compared using a *t*-test.

The field survey identified three common characteristics of wild plants, 1) undisturbed plants; 2) plants whose culms were damaged or broken; 3) and plants that were entirely submersed in deeper water.

The greenhouse study was designed to simulate these three conditions.

Nine fiberglass fish culture tanks, measuring, 0.61 m x 3.1 m, were set up in the greenhouse located at the San Marcos Fish Hatchery and Technology Center. Water was pumped into tanks at a rate of 2 gal/min. with a re-circulation system. Water temperature was maintained in the tanks at 22°C using heater/chiller units.

Plants were grown from seed and individual seedlings were planted in a 1.6 liter pots. A clipped treatment simulated herbivory or broken culms. A submerged treatment simulated plants growing in deeper, swifter water where disturbance by water fowl and recreationists is less common. For the clipped treatment, culms were allowed to emerge and were clipped at the water's surface five days after emergence. For the submerged treatment, culms and leaves were held below the water surface with 0.6 cm diameter bamboo frames. Control plants were not manipulated in any way. There were nine replicate tanks, each containing three plants; one plant in each tank was assigned to each treatment (i.e. clipped, control, submerged) for a total of nine replicate plants for each treatment. Plants were placed in tanks one month prior to the start of the study to allow for acclimation and growth of culms. The number of reproductive culms and tillers for each plant were counted and recorded at 4, 8, 12, 16, and 20 weeks. At the end of 20 weeks, plants were harvested, dried, and their weight recorded.

A repeated measures ANOVA was used to determine significant differences in numbers of culms and tillers between treatments. A single-factor ANOVA was used to determine significant differences in biomass between treatments ($\alpha=0.05$).

RESULTS

We randomly selected forty-eight of the 137 30.5 m sections of the river in which *Z. texana* occurred. Twenty-two transects with 37 quadrats intersected *Z. texana* and were sampled.

Mean water depth of quadrats was 0.77 m (± 0.24) with a range of 0.33 m to 1.28 m. These results are in agreement with the Poole and Bowles (1999) habitat assessment. Mean current velocity was 0.13 m/s (± 0.16) with a range of 0 m/s to 0.74 m/s. These values are considerably lower than the Poole and Bowles (1999) study who reported the mean current velocity for *Z. texana* stands to be >0.46 m/s.

Table 5-1 reports the means for data collected from all quadrats. Reproductive culms were present in 32 of 37 quadrats. Broken culms, shredded culms, and submerged inflorescences represented 53.6% of total culms and will not produce seed. Culms in the seed-producing stage were considered reproductive and represented 1% of total culms.

Emergent culms and submersed, developing culms were considered potentially reproductive and represented 45.4% of total culms.

Floating mats were present in nine of 37 (24.3%) quadrats. The mats covered 67% ($\pm 29\%$, range 15%-100%) of the quadrat. There were significantly more broken culms in quadrats with vegetation mats (mean = 4.8 culms; $p < 0.05$) compared with quadrats

Table 5-1. Mean, standard deviation, and range of values for *Zizania texana* culms in randomly selected quadrats (25 cm X 25 cm); n = 37.

Culms	Mean	SD	Range
Total culms	8.03	6.36	0-23
Total tillers	0.07	2.59	0-8
Developing culms, submersed	3.57	3.58	0-11
Broken culm, terminal inflorescence will not form	2.95	2.79	0-10
Shredded culm	0.68	2.11	0-11
Submerged inflorescence	0.68	1.42	0-5
Emergent culms	0.08	0.36	0-2
Seed-producing stage	0.08	0.36	0-2

without vegetation mats (mean = 2.4 culms). There was no significant difference in the remaining culm categories in quadrats with vegetation mats compared to quadrats without vegetation mats.

The majority of quadrats had vegetation on all sides. Only two quadrats were surrounded by bare ground. *Zizania texana* was the only neighbor in 25 quadrats. Other native plants were the neighbor in six quadrats and exotic species were the neighbor in four quadrats.

In the greenhouse study, plants in all treatments produced culms and tillers but there were no significant differences between treatments. The treatment with the greatest number of culms at week 20 was the submerged treatment with 10.0 (± 4.2) followed by the control and clipped treatment (8.0 ± 3.3 and 5.9 ± 2.5 respectively).

Most, but not all plants produced asexual tillers. The treatment with the greatest number of tillers was the clipped treatment with 9.0 ± 5.9 followed by control and submerged (7.3 ± 8.7 and 7.1 ± 5.1 , respectively). The number of tillers per culm was also similar among treatments. The clipped treatment produced 1.5 tillers per culm, control plants produced 0.91 tillers per culms, and the submerged treatment produced 0.71 tillers per culm.

There was no significant difference in culm or tiller biomass between treatments. Submerged treatment had the greatest culm biomass followed by control and clipped treatments (18.3 ± 10.0 ; 15.7 ± 4.3 ; and 10.0 ± 7.4 , respectively). Submerged treatment had the greatest tiller biomass followed by clipped and control treatments (12.7 ± 7.0 , 11.1 ± 8.7 ; and 10.9 ± 9.3 , respectively).

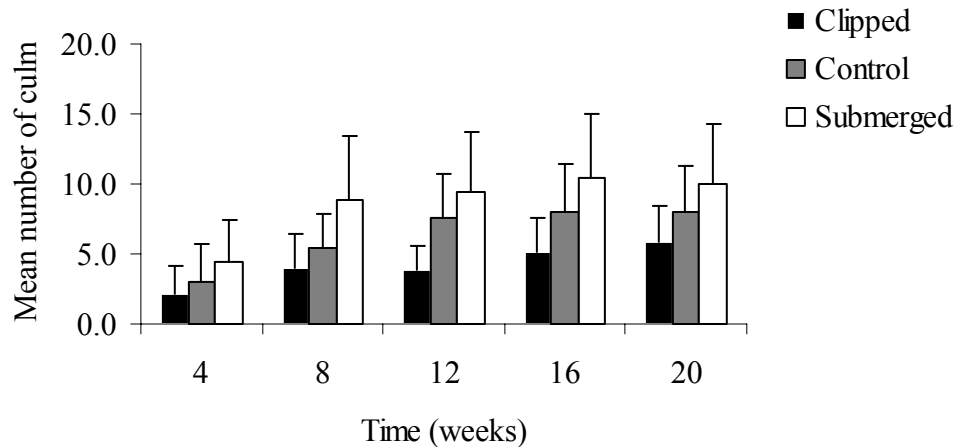


Figure 5-1. Mean number of *Zizania texana* culms produced during the greenhouse study. There were no significant differences among treatments. The mean number of culms per plant at week 16 and 20 were similar to the mean number of culms in 0.065 m² quadrats in the field survey.

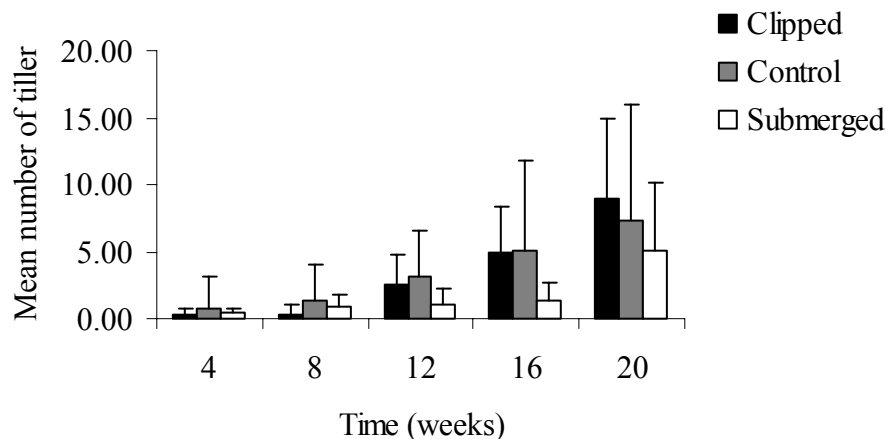


Figure 5-2. Mean number of *Zizania texana* tillers produced during greenhouse study. There were no significant difference among treatments. At week 20, the mean number of tillers per culm was 1.1 for all study plants.

DISCUSSION

Mean current velocity was 0.13 m/s (± 0.16) with a range of 0 to 0.74 m/s. These values are considerably lower than Poole and Bowles (1999) who reported the mean current velocity for *Z. texana* stands to be >0.46 m/s. Poole and Bowles sampled current velocity upstream from *Z. texana* where current was not obstructed by vegetation in the water column. In this study, current velocity was sampled immediately upstream of each quadrat and reflects the velocity encountered by *Z. texana* within a stand. The values for current velocity within macrophyte beds are relatively slow because the flow of water is obstructed by vegetation (Losee and Wetzel, 1993). The highest value for current velocity

in this survey, 0.74 m/s, was recorded for the two quadrats at the leading edge of a stand of *Z. texana* with no other vegetation present. This value is in agreement with findings from Poole and Bowles (1999).

The flowering period for *Z. texana* is April to November (Gould, 1975). Values for culms were recorded during the last two weeks in May; thus, we believe the survey accurately reflects reproductive activity during 2002. Reproductive failure, long considered a threat to the species (Emery, 1967; 1977; U.S. Fish and Wildlife Service, 1996), was found in 53.6% of all culms in the survey (Table 5-2). Some culms were broken or shredded and this type of damage can be attributed to vegetation mats floating over *Z. texana* and interfering with culm emergence or directly damaging culms (Power, 1996). It also is possible that waterfowl swimming in or feeding on *Z. texana* damages culms. Some inflorescences were submerged.

Zizania texana is wind pollinated and successful pollination will not occur under water. Furthermore, fertilized ovules will not develop into viable seeds when submerged (Power, 1997). Moving water may play a role in knocking down maturing inflorescences; however, there was no significant relationship between current velocity and submerged inflorescences. Wind, waterfowl, or recreational users of the river also may play a role in submerging mature inflorescences. The relationship between recreational use of the river and reproductive failure is unclear, especially since Sewell Park is the only area of the river that has had successful seed production in the past 40 years and is also one of the top recreational areas of the river (Bradsby, 1994; Breslin, 1997), with thousands of people swimming, canoeing, kayaking, and wading in and around *Z. texana*.

Table 5-2. The reproductive potential of *Zizania texana* culms in randomly selected quadrats in the San Marcos River; n=37

Culm	%	Reproductive Potential	Comments
Seed-producing culm	1.0	Reproductive success	Recruitment unknown
Emergent culm	1.0	Potentially reproductive	Vulnerable to factors that result in reproductive failure
Submersed, developing culm	44.4		
Submerged inflorescence	8.5	Reproductive failure	
Shredded culm	8.5		Most likely the result of extrinsic factors
Broken culm	36.7		

Forty-four percent of developing culms were submersed and potentially reproductive. All developing culms are vulnerable to breaking, shredding or being submerged after floral development. Two percent of culms were emergent. Using the results of the survey, *Z. texana* produces about three emergent culms per m². This culm density is not enough for successful sexual reproduction to occur (see Chapter 3). Identifying and eliminating the

factors that result in broken or shredded culms and submerged inflorescences would result in more emergent culms per m² and increase the chances of successful sexual reproduction.

Zizania texana has a patchy distribution and stands are described as “fragmented.” In this survey, 5% of plants sampled were smaller than 0.0625 m² (25 cm X 25 cm) at ground level and were surrounded by bare ground. Ninety-five percent of quadrats had neighboring plants. Sixty-eight percent of quadrats were surrounded by other *Z. texana* individuals. The remaining 27.4 % of quadrats had native and nonnative species as neighbors. *Hygrophila polysperma* was the only neighboring exotic species and *Potamogeton illinoensis*, *Ceratophyllum demersum*, and *Hydrocotyle umbellata* were the only native neighbors. In the Saunders et al (2001) study on the San Marcos River, 34.29% of sample cells contained *P. illinoensis* in association with *Z. texana*. In this survey 18.9% of quadrats had *P. illinoensis* as a neighbor on at least one side, 10.1% had *H. polysperma* as a neighbor, 2.7% had either *C. demersum* or *H. umbellata*. Notably missing from the survey was *Hydrilla verticellata*. In the Saunders et al (2001) study, 72.86% of sample cells containing *Z. texana* had *H. verticellata* associated with them and 50% of cells had *H. polysperma* associated with them. The difference in neighbors may be explained by the fact that sampling in the Saunders et al. (2001) study took place prior to the floods of 1998 and 2001 and this survey was conducted after those flood events. It is possible the abundance and distribution of some species was significantly altered by the flood events. In a study of the effects of 1998 flood on vegetation in the Comal River in Comal County, Texas, Robert Doyle found that *Hydrilla* and *Hygrophila* cover increased after flooding relative to native plant species (*pers. comm.*, Dr. Robert Doyle, Professor, Baylor University). In contrast, BIO-WEST, Inc. found a reduction in *Hydrilla* and *Hygrophila* after the 2002 flood in the San Marcos River. The impact of flooding on native and non-native vegetation distribution and abundance requires further study.

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APPENDICES

Appendix 1-1. *Zizania texana* pollination study raw data. Number of inflorescences setting seeds for each treatment. Treatments: no treatment (Control), anthers removed (Emasculate), within-panicle self-pollination (WIG), within-panicle hand self-pollination (WIHG), between-panicle self-pollination (BG), and between-panicle hand self-pollination (BHG).

Treatment	Control	Emasculate	WIG	WIHG	BG	BHG	Total
Seed set	9	5	2	4	6	3	29
No seed set	1	5	8	5	3	6	28
Total	10	10	10	9	9	9	57

Appendix 1-2. *Zizania texana* pollen viability study raw data. Seventy-six samples of pollen were collected from a population of plants May 15 – August 17, 2002. Each pollen sample was divided into seven 10-minute subsamples and each subsample was tested for percent germination. A minimum of 100 pollen grains was counted for each sample.

Time (minutes)	0	10	20	30	40	50	60
9.7	0	0	0	0	0.2	0	0
5.5	16.2	0	0	0	0	0	0
39	0.6	0	0	0	0	0	0
1.4	2.4	0.4	0.2	0.4	1.2	0	
24	0	0	0	0	0	0	0
0.94	1.5	0	0	0	0	0	0
4	0	0	0	0	0	0	0
7.9	0	0	0	0	0	0	0
0.4	0	0	0	0	0	0	0
7.1	0	0	0	0	0	0	0
12.3	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0.4	0	0	0	1.8	0	0	0
7.7	0	0	0	0	0	0	0
1.6	0	0	0	0	0	0	0
29.1	1.99	0	32	0	0	21	
0.54	0	0	0	0	0	0	0
2.49	0	0	0	50	0	0	0
2.3	0	0	0	0	0	0	0
1.4	8.7	5.2	2.5	1.7	2.8	2.1	
45	2.2	0	0	0	0	0	0
14	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0
1.9	0	0	0	0	0	0	0
18	0	0	0	0.8	0	0	0
2.4	0	0	0	0	0	0	0
1.1	0	0	0	0	0	0	0
5.5	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
2.5	0	0	0	0	0	0	0
3.1	0	0	0	0	0	0	0
4.1	0	0	0.3	0	0	0	0
14	0	0	0	0	0	0	0
12	0.8	0	0	0	0	0	0
21	0.5	0	0	0	0	0	0
1.9	0.6	0.4	0	0	0	0	0

Appendix 1-2 (continued). *Zizania texana* pollen viability study raw data. Seventy-six samples of pollen were collected from a population of plants May 15 – August 17, 2002. Each pollen sample was divided into seven 10-minute subsamples and each subsampled was tested for percent germination. A minimum of 100 pollen grains was counted for each sample.

Time (minutes)	0	10	20	30	40	50	60
2.8	0	0	0	0	0	0	0
0.7	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0.7	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
7.2	1.8	2.2	3.4	3.9	4.2	4.4	
11	1.5	0	0	0	0	0	0
22	0	0	0	0	0	0	0
26.8	0	0	0	0	0	0	0
10.9	0	0	0	0	0	0	0
6.9	0.7	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0.57	0	1.5	0	0	0	0	0
0.8	0	0	0	0	0	0	0
0	1.6	0	0	0	0	0	0
0	0	0	0	0	0	0	0
12.2	0	0	0	0	0	0	0
20	0	0.9	0	0	0	0	0
17.1	0	0	0	0	0	0	0
3.2	0	0	0	0	0	0	0
13.2	0	0	0	0	0	0.7	0
12.4	0	0	0	0	0	0	0
3.8	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
7.4	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
17	1.2	0	0	0	0	0	0
43.3	0	0	0	0	0	0	0
21.6	0	0	0	0	0	0	0
28.2	0.55	0	0	0	0	0	0
1.7	0	0	0	0	0	0	0
1.2	0.61	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0							
0.55							
0							
18.4							
2.2							

Appendix 1-3. *Zizania texana* stigma receptivity study raw data. Study took place May 29 – August 5, 2002. Receptive stigmas were scored as “+” and nonreceptive stigmas were scored as “-”.

Day								
	1	2	3	4	5	6	7	8
	+	+	+	+	-	-	-	-
	+	+	+	+	-	+	-	-
	+	+	+	-	+	+	-	-
	+	+	+	-	-	+	-	-
	-	+	-	+	+	-	-	-
	-	+	+	-	-	-	-	-
	+	+	+	-	+	+	-	-
	+	-	-	-	-	-	-	-
	+	-	-	+	+	-	-	-
	+	+	-	-	-	+	-	-
	+	+	-	-	-	+	-	-
	+	-	+	+	-	+	-	-
	+	+	+	-	-	-	-	-
	+	-	-	-	-	-	-	-
	+	+	-	+	+	-	+	-
	+	+	+	+	+	+	+	-
	+	+	+	+	+	-	+	+
	+	+	+	-	-	-	-	+
	+	+	+	+	+	-	+	+
	-	+	+	+	-	+	+	-

Appendix 1-4. Chi-square analysis of pollination study data. Treatments: no treatment (Control), anthers removed (Emasculate), within-panicle self-pollination (WIG), within-panicle hand self-pollination (WIHG), between-panicle self-pollination (BG), and between-panicle hand self-pollination (BHG).

Treatment	Control		Emasculate		WIG		WIHG		BG		BHG		Total
Frequency	Observed	(f)	Observed	(f)	Observed	(f)	Observed	(f)	Observed	(f)	Observed	(f)	
Seed set	9	5.08	5	5.08	2	5.08	4	4.57	6	4.57	3	4.57	29
No seed set	1	4.91	5	4.91	8	4.91	5	4.42	3	4.42	6	4.42	28
Total	10		10		10		9		9		9		57

$\chi^2_{\text{test}} = 12.079$ Observed and expected frequency (f) values are reported for each cell. Significance at $\alpha = 0.05$.

$\chi^2_{\text{crit}} = 11.07$ (5 degrees of freedom)

$\chi^2_{\text{test}} > \chi^2_{\text{crit}}$

Reject H_0

Appendix 1-5. Multiple comparison analysis of pollination study data using a non-parametric Tukey multiple comparison test ($\alpha = 0.05$). Treatments: no treatment (Control), anthers removed (Emasculate), within-panicle self-pollination (WIG), within-panicle hand self-pollination (WIHG), between-panicle self-pollination (BG), and between-panicle hand self-pollination (BHG).

Treatment Comparison	q_{test} value	$q_{\text{crit}} = 4.03$
Control vs Emas	2.54	
Control vs WIG	4.46*	
Control vs WIHG	3.18	
Control vs BG	1.9	
Control vs BHG	3.82	
Emas vs WIG	1.91	
Emas vs WIHG	0.637	
Emas vs BG	0.637	
Emas vs BHG	1.27	
WIG vs WIHG	1.27	
WIG vs BG	2.54	
WIG vs BHG	0.637	
WIHG vs BG	1.27	
WIHG vs BHG	0.637	
BG vs HBG	0.637	

* indicates significant difference between q_{test} and q_{cri} .

Appendix 1-6. Chi-square analysis of pollen germination comparing the factor of time to pollen germination.

Time	0		10		20		30		40		50		60		Total
Frequency	Observed (f)		Observed (f)		Observed (f)		Observed (f)		Observed (f)		Observed (f)		Observed (f)		
Germination	63	16.05	18	14.99	6	14.99	5	14.99	7	14.99	4	14.99	3	14.99	106
No germination	13	59.95	53	56.00	65	56.00	66	56.00	64	56.00	67	56.00	68	56.00	396
Total	76		71		71		71		71		71		71		502

$\chi^2_{\text{test}} = 217.905$ Observed and expected frequency (f) values are reported for each cell. Significance at $\alpha = 0.05$.

$\chi^2_{\text{crit}} = 12.59$ (6 degrees of freedom)

$\chi^2_{\text{test}} > \chi^2_{\text{crit}}$

Reject H_0

Appendix 1-7. Multiple comparison analysis of pollen germination study data using a non-parametric Tukey multiple comparison test ($\alpha = 0.05$).

Time Interval Comparison	q_{test} value	$q_{\text{crit}} = 4.17$
0 vs 10 minutes	10.61*	
0 vs 20	13.44*	
0 vs 30	13.68*	
0 vs 40	13.21*	
0 vs 50	13.92*	
0 vs 60	14.15*	
10 vs 20	2.83	
10 vs 30	3.07	
10 vs 40	2.59	
10 vs 50	3.30	
10 vs 60	3.54	
20 vs 30	0.236	
20 vs 40	0.236	
20 vs 50	0.472	
20 vs 60	0.708	
30 vs 40	0.472	
30 vs 50	0.236	
30 vs 60	0.472	
40 vs 50	0.708	
40 vs 60	0.708	
50 vs 60	0.236	

* indicates significant difference between q_{test} and q_{cri} .

Appendix 1-8. *Zizania texana* stigma receptivity data analysis. Receptive stigmas were scored as “1” and nonreceptive stigmas were scored as “0”.

	Day							
	1	2	3	4	5	6	7	8
1	1	1	1	1	0	0	0	0
1	1	1	1	1	0	1	0	0
1	1	1	1	0	1	1	0	0
1	1	1	1	0	0	1	0	0
0	1	0	1	1	1	0	0	0
0	1	1	1	0	0	0	0	0
1	1	1	1	0	1	1	0	0
1	0	0	0	0	0	0	0	0
1	0	0	1	1	1	0	0	0
1	1	0	0	0	0	1	0	0
1	1	0	0	0	0	1	0	0
1	0	1	1	1	0	1	0	0
1	1	1	1	0	0	0	0	0
1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
1	1	0	1	1	1	0	1	0
1	1	1	1	1	1	1	1	0
1	1	1	1	1	1	0	1	1
1	1	1	1	0	0	0	0	1
1	1	1	1	1	1	0	1	1
0	1	1	1	1	0	1	1	0
Total	18.00	16.00	13.00	10.00	8.00	9.00	5.00	3.00
Mean	0.86	0.76	0.62	0.48	0.38	0.43	0.24	0.14
S Dev	0.36	0.44	0.50	0.51	0.50	0.51	0.44	0.36

Appendix 1-9. Multiple comparison analysis of stigma receptivity study data using a non-parametric Tukey multiple comparison test ($\alpha = 0.05$).

Time Interval Comparison	q_{test} value	$q_{\text{crit}} = 4.29$
Day 1 vs 2	0.870	
1 vs 3	2.18	
1 vs 4	3.48	
1 vs 5	4.35*	
1 vs 6	3.92	
1 vs 7	5.66*	
1 vs 8	6.53*	
2 vs 3	1.31	
2 vs 4	2.61	
2 vs 5	3.48	
2 vs 6	3.05	
2 vs 7	4.79*	
2 vs 8	5.66*	
3 vs 4	1.31	
3 vs 5	2.18	
3 vs 6	1.74	
3 vs 7	3.48	
3 vs 8	4.35*	
4 vs 5	0.870	
4 vs 6	0.435	
4 vs 7	2.18	
4 vs 8	3.05	
5 vs 6	0.435	
5 vs 7	1.31	
5 vs 8	2.18	
6 vs 7	1.74	
6 vs 8	2.61	
7 vs 8	0.870	

* indicates significant difference between q_{test} and q_{cri} .

Appendix 1-10. Air temperature, percent relative humidity, and wind speed during study period, May 15 – August 17.

Date	Time a.m.	Air temp mean °C	% Relative Humidity mean	Wind Speed meters/sec
15-May	0600	13.25	95.40	0.563
15-May	0700	12.73	96.50	0.889
16-May	0600	23.23	88.20	2.725
16-May	0700	23.38	88.30	2.677
17-May	0600	20.25	88.30	3.758
17-May	0700	19.95	90.90	1.475
18-May	0600	17.10	66.23	4.586
18-May	0700	16.31	69.50	4.346
19-May	0600	12.31	72.70	2.161
19-May	0700	12.26	71.80	2.761
20-May	0600	10.43	72.60	1.702
20-May	0700	10.37	73.40	1.657
21-May	0600	12.96	90.70	1.102
21-May	0700	12.34	90.60	1.129
22-May	0600	14.55	95.00	0.804
22-May	0700	13.88	96.50	1.397
23-May	0600	20.87	88.00	3.097
23-May	0700	20.85	88.40	2.863
24-May	0600	20.78	89.90	2.094
24-May	0700	21.30	88.10	2.49
25-May	0600	20.86	91.10	1.18
25-May	0700	20.97	92.60	2.355
26-May	0600	21.46	72.60	5.32
26-May	0700	21.32	87.70	4.375
27-May	0600	20.13	95.40	0.875
27-May	0700	19.69	97.00	0.409

Appendix 1-10 (continued)

Date	Time a.m.	Air temp mean °C	% Relative Humidity mean	Wind Speed meters/sec
28-May	0600	16.96	97.9	1.656
28-May	0700	17.16	95.7	2.445
29-May	0600	20.79	93.7	1.114
29-May	0700	20.52	95.4	1.114
30-May	0600	19.96	94.3	1.102
30-May	0700	19.81	93.9	0.384
31-May	0600	19.41	90.1	2.259
31-May	0700	19.53	90.7	2.37
01-Jun	0600	18.47	92.2	1.272
01-Jun	0700	18.39	92.9	1.452
02-Jun	0600	20.12	94.7	0.129
02-Jun	0700	21.05	93.9	0.548
03-Jun	0600	21.87	95.4	0.771
03-Jun	0700	22.01	95.7	0.796
04-Jun	0600	24.15	90.9	2.446
04-Jun	0700	23.86	92.4	2.082
05-Jun	0600	23.74	90.3	1.663
05-Jun	0700	23.56	90.3	0.832
06-Jun	0600	23.01	79.2	2.016
06-Jun	0700	22.94	78.5	2.208
07-Jun	0600	21.82	85.9	0.985
07-Jun	0700	21.51	87.8	0.915
08-Jun	0600	22.95	94	0.751
08-Jun	0700	22.99	94.6	0.696
09-Jun	0600	24.79	91.0	1.994
09-Jun	0700	24.89	90.8	2.186

Appendix 1-10 (continued)

Date	Time a.m.	Air temp mean °C	% Relative Humidity mean	Wind Speed meters/sec
10-Jun	0600	25.24	86.9	2.87
10-Jun	0700	25.26	87.1	2.836
11-Jun	0600	24.59	91.2	1.936
11-Jun	0700	24.09	93.9	1.117
12-Jun	0600	23.77	94.7	0.98
12-Jun	0700	23.95	95.4	1.017
13-Jun	0600	23.16	92.4	0.779
13-Jun	0700	23.24	92.7	0.408
14-Jun	0600	23.03	92.3	0.123
14-Jun	0700	21.84	94.5	1.075
15-Jun	0600	23.36	68.35	1.132
15-Jun	0700	22.62	68.01	1.412
16-Jun	0600	18.91	95.2	3.572
16-Jun	0700	18.91	95.6	2.748
17-Jun	0600	19.43	89.6	1.299
17-Jun	0700	19.33	90.1	1.173
18-Jun	0600	18.68	87.9	0.51
18-Jun	0700	18.06	91.1	1.007
19-Jun	0600	20	95.1	0.632
19-Jun	0700	19.27	96.8	0.152
20-Jun	0600	23.74	94.9	0.113
20-Jun	0700	23.75	95.8	1.406
21-Jun	0600	21.79	96	0.638
21-Jun	0700	21.41	96.6	0.547
22-Jun	0600	21.48	93.8	0.865
22-Jun	0700	21.07	95.5	1.182
23-Jun	0600	18.44	90.3	1.647
23-Jun	0700	18.8	91.8	0.59

Appendix 1-10 (continued)

Date	Time a.m.	Air temp mean °C	% Relative Humidity mean	Wind Speed meters/sec
24-Jun	0600	20.63	82.7	0.135
24-Jun	0700	20.09	84.9	0.063
25-Jun	0600	20.16	93.2	1.152
25-Jun	0700	20.21	93.2	0.716
26-Jun	0600	21.54	97.2	0.178
26-Jun	0700	21.63	97.4	0.19
27-Jun	0600	20.01	93.3	1.191
27-Jun	0700	19.15	96.1	0.624
28-Jun	0600	22.58	93.3	0.285
28-Jun	0700	22.54	93.8	0.015
29-Jun	0600	23.05	94.6	0.775
29-Jun	0700	22.74	95.6	0.745
30-Jun	0600	21.76	98.7	3.638
30-Jun	0700	22.06	98.6	4.193
01-Jul	0600	24.33	96.6	3.246
01-Jul	0700	21.93	92.6	1.501
02-Jul	0600	24.08	97	2.271
02-Jul	0700	21.04	97.5	4.113
03-Jul	0600	24.79	94.3	3.607
03-Jul	0700	23.64	89	2.936
04-Jul	0600	25.03	88.5	4.143
04-Jul	0700	25.06	87.5	3.871
05-Jul	0600	23.69	94.2	4.161
05-Jul	0700	22.45	96.2	3.727
06-Jul	0600	21.8	98.1	0.038
06-Jul	0700	21.58	98.6	0.235

Appendix 1-10 (continued)

Date	Time a.m.	Air temp mean °C	% Relative Humidity mean	Wind Speed meters/sec
07-Jul	0600	22.86	97.8	0.071
07-Jul	0700	22.2	98	0.272
08-Jul	0600	22.6	94.3	1.024
08-Jul	0700	22.33	95.3	0.753
09-Jul	0600	23.11	94.7	0.607
09-Jul	0700	22.94	94.4	1.013
10-Jul	0600	23.1	97	0.065
10-Jul	0700	23.06	96.6	0.383
11-Jul	0600	22.78	96.6	0.903
11-Jul	0700	22.68	97.6	0.089
12-Jul	0600	23.48	82.4	1.512
12-Jul	0700	22.59	87.7	0.825
13-Jul	0600	24.69	85.7	2.518
13-Jul	0700	24.24	87.9	0.537
14-Jul	0600	22.68	95.5	0.001
14-Jul	0700	22.42	96.3	0.172
15-Jul	0600	22.11	96.6	0.91
15-Jul	0700	22.05	96.4	0.829
16-Jul	0600	23.14	98.1	1.215
16-Jul	0700	23.25	97.5	1.57
17-Jul	0600	23.23	96.3	1.463
17-Jul	0700	23.32	96.1	1.098
18-Jul	0600	24.25	96.6	0.945
18-Jul	0700	23.85	97.4	1.047
19-Jul	0600	24.1	94.7	2.748
19-Jul	0700	23.73	94.5	2.288
20-Jul	0600	24.39	94.2	1.64
20-Jul	0700	24.1	94.9	2.121

Appendix 1-10 (continued)

Date	Time a.m.	Air temp mean °C	% Relative Humidity mean	Wind Speed meters/sec
21-Jul	0600	23.97	95.6	1.46
21-Jul	0700	24.41	95.4	2.097
22-Jul	0600	23.36	97.1	1.944
22-Jul	0700	23.49	96.5	2.14
23-Jul	0600	22.64	97.7	1.102
23-Jul	0700	22.4	97.8	0.558
24-Jul	0600	22.73	96.3	0.9
24-Jul	0700	22.47	97.5	0.158
25-Jul	0600	21.77	94.4	1.242
25-Jul	0700	21.57	94.5	0.267
26-Jul	0600	23.77	96.4	2.242
26-Jul	0700	23.8	95.6	2.206
27-Jul	0600	25.08	90.1	3.864
27-Jul	0700	24.69	92.2	2.895
28-Jul	0600	25.05	91.4	2.469
28-Jul	0700	24.53	93.2	1.796
29-Jul	0600	25.67	88.7	2.636
29-Jul	0700	25.46	89.9	2.29
30-Jul	0600	25.42	92.5	2.23
30-Jul	0700	24.98	94.3	2.043
31-Jul	0600	25.29	87.4	4.482
31-Jul	0700	25.14	89	3.325
01-Aug	0600	23.12	94.8	2.041
01-Aug	0700	22.64	96	1.413
02-Aug	0600	23.89	96.3	0.445
02-Aug	0700	23.11	97.5	0.139

Appendix 1-10 (continued)

Date	Time a.m.	Air temp mean °C	% Relative Humidity mean	Wind Speed meters/sec
03-Aug	0600	22.4	94.7	0.358
03-Aug	0700	21.63	96.2	0.302
04-Aug	0600	21.1	95.9	0.937
04-Aug	0700	20.71	96.8	0.885
05-Aug	0600	22.04	90.4	0.628
05-Aug	0700	22.16	89.8	0.568
06-Aug	0600	22.34	91.9	0.027
06-Aug	0700	22.29	91.7	0.111
07-Aug	0600	23	85.8	0.748
07-Aug	0700	22.87	86.6	0.557
08-Aug	0600	24.05	80.4	1.592
08-Aug	0700	24.39	80.4	1.563
09-Aug	0600	24.48	89.6	0.988
09-Aug	0700	24.23	91.4	0.477
10-Aug	0600	22.82	95.6	0.093
10-Aug	0700	22.44	96.6	0.682
11-Aug	0600	20.84	97.8	0.821
11-Aug	0700	20.47	98.1	0.979
12-Aug	0600	22.77	95.7	0.121
12-Aug	0700	23.11	95.3	0.054
13-Aug	0600	24.76	90.7	1.958
13-Aug	0700	24.24	94.2	1.69
14-Aug	0600	24.14	94.9	1.061
14-Aug	0700	23.64	96.6	1.549
15-Aug	0600	25.28	89.7	3.321
15-Aug	0700	25.37	88.7	3.956
16-Aug	0600	25	94	1.702
16-Aug	0700	25.35	93.7	1.71

Appendix 1-10 (continued)

Date	Time a.m.	Air temp mean °C	% Relative Humidity mean	Wind Speed meters/sec
17-Aug	0600	25.93	90.6	1.776
17-Aug	0700	25.4	93.1	1.544

Appendix 1-11. Daily average , maximum, and minimum air temperature, % relative humidity, and wind speed during the study period, May 15 – August 17, 2002.

Date	Ave Air Tmp (°C)	Max Air Tmp (°C)	Min Air Tmp (°C)	Ave RH (%)	Max RH (%)	Min RH (%)	Ave WS (mps)	Max WS (mps)	Min WS (mps)
5/15	19.49	25.95	12.96	53.81	77.8	29.81	4.69	6.95	2.432
5/16	21.95	30.59	12.26	67.2	97.6	36.8	5.61	8.45	2.761
5/17	26.66	33.38	21.27	67.79	94	41.58	6.78	9.8	3.762
5/18	24.79	31.48	19.57	70.99	92.8	49.18	8.48	13.4	3.564
5/19	20.56	26.94	15.59	47.84	73.2	22.48	8.80	12.5	5.095
5/20	18.35	26.41	11.64	46.74	75.6	17.89	4.83	7.25	2.403
5/21	18.9	27.44	9.94	50.66	75.4	25.91	4.55	6.8	2.3
5/22	20.96	28.84	12.07	64.04	94.2	33.87	6.43	9.8	3.051
5/23	22.73	31.05	13.66	65.6	97.4	33.79	7.45	11.15	3.736
5/24	24.79	30.99	20.54	64.26	89.5	39.02	8.18	11.75	4.602
5/25	23.54	27.68	20.1	75.29	91.7	58.88	5.64	8.3	2.983
5/26	25.75	32.48	20.14	66.54	94.2	38.89	4.40	6.65	2.154
5/27	26.16	32.88	19.6	65.28	92.6	37.96	7.81	12.8	2.821
5/28	25.67	32.82	19.1	66.4	97.9	34.89	6.65	10.1	3.198
5/29	24.08	30.07	16.65	73.56	98.6	48.52	6.76	11	2.522
5/30	23.94	32.08	18.12	74.24	97.3	51.17	7.12	12.05	2.192
5/31	26.64	35.91	18.84	60.49	98.6	22.37	5.07	8.3	1.836
6/1	28.01	31.85	22.5	55.65	72.9	38.39	5.50	8	2.999
6/2	25.51	31.82	18.11	67.21	93.9	40.52	3.77	5.75	1.779
6/3	25.77	31.98	19.27	70.66	96	45.31	4.57	6.95	2.179
6/4	26.71	33.21	21.7	70.58	97.4	43.75	5.56	8.6	2.506
6/5	27.88	34.01	23.7	65.55	93.1	37.99	6.52	9.65	3.382
6/6	28.19	34.18	23.2	62.63	92.7	32.56	5.08	8	2.146

Appendix 1-11 (cont.). Daily average , maximum, and minimum air temperature, % relative humidity, and wind speed during the study period, May 15 – August 17, 2002.

Date	Ave Air Tmp (°C)	Max Air Tmp (°C)	Min Air Tmp (°C)	Ave RH (%)	Max RH (%)	Min RH (%)	Ave WS (mps)	Max WS (mps)	Min WS (mps)
6/7	28.42	34.05	22.64	58	80.5	35.49	7.94	7.1	2.765
6/8	27.69	34.08	21.27	65.41	88.7	42.12	5.15	8.45	1.836
6/9	27.85	34.61	22.57	64.85	95.5	34.19	5.31	8.3	2.318
6/10	27.97	33.39	24.23	69.94	92.9	46.98	7.44	11.45	3.429
6/11	28.82	35.21	24.62	63.61	89.8	37.42	6.20	8.75	3.652
6/12	28.9	35.74	23.87	63.83	94.4	33.26	5.29	7.7	2.883
6/13	28.8	35.94	23.5	66.38	95.7	37.06	5.72	8.9	2.531
6/14	28.5	34.81	22.87	64.43	94	34.86	4.74	7.55	1.927
6/15	28.96	35.78	21.47	66.45	95.5	37.39	4.87	7.55	2.191
6/16	27.74	34.18	22.24	62.96	88.6	37.33	4.72	7.1	2.325
6/17	25.61	32.55	18.6	69.61	97.3	41.92	8.81	14.15	3.458
6/18	25.65	32.78	19.05	61.08	91.1	31.06	4.02	6.2	1.835
6/19	26	33.18	17.66	62.32	93	31.63	4.68	7.25	2.103
6/20	27.44	35.11	18.84	65.98	97.6	34.36	5.06	7.85	2.266
6/21	28.01	33.91	23.47	66.78	96.2	37.36	6.58	10.4	2.764
6/22	28.04	34.45	21.1	64.02	97	31.03	4.47	7.25	1.686
6/23	27.94	34.68	20.7	62.59	96	29.17	4.18	6.65	1.698
6/24	27.27	34.65	17.92	59.55	93.6	25.5	4.33	7.25	1.409
6/25	27.8	34.95	19.61	58.07	87.4	28.73	5.16	8.45	1.872
6/26	27.33	36.58	19.9	62.52	95	30.03	7.53	13.25	1.802
6/27	26.7	36.44	18.54	65.92	98	33.83	7.68	13.4	1.945

Appendix 1-11 (cont.). Daily average , maximum, and minimum air temperature, % relative humidity, and wind speed during the study period, May 15 – August 17, 2002.

Date	Ave Air Tmp (°C)	Max Air Tmp (°C)	Min Air Tmp (°C)	Ave RH (%)	Max RH (%)	Min RH (%)	Ave WS (mps)	Max WS (mps)	Min WS (mps)
6/28	25.36	32.09	18.91	70.88	97.2	44.55	4.65	7.1	2.194
6/29	25.5	33.48	22.07	70.48	94.9	46.05	7.14	12.65	1.616
6/30	23.77	27.01	22.17	83.85	97.7	70	4.65	6.8	2.488
7/1	23.32	29.14	20.47	83.51	98.9	68.11	8.80	14.9	2.702
7/2	23.1	24.6	20.84	90.8	98.4	83.2	4.58	7.25	1.913
7/3	23.21	24.43	19.14	92.35	99.2	85.5	6.11	9.8	2.406
7/4	26.3	32.23	21.1	72.48	97.9	47.05	7.56	11.6	3.506
7/5	26.85	32.32	23.53	68.45	90.7	46.19	6.88	10.4	3.347
7/6	25.54	30.61	21.67	81.61	97.5	65.71	7.20	12.05	2.344
7/7	26.73	32.88	21.1	75.41	98.9	51.91	3.79	6.2	1.378
7/8	26.43	33.35	21.57	73.39	98.6	48.18	5.79	10.4	1.182
7/9	27.95	34.88	22.14	68.91	96.5	41.32	6.08	10.4	1.761
7/10	26.39	33.15	22.67	72.21	96.2	48.21	6.89	11.9	1.878
7/11	26.95	33.31	22.67	69.69	97.7	41.68	3.79	6.2	1.377
7/12	27.35	34.21	22.2	72.03	98.1	45.95	3.93	6.65	1.207
7/13	28.67	35.68	22.27	63.41	89.3	37.52	3.28	5	1.561
7/14	24.84	29.41	22.41	81.67	95.2	67.14	4.36	6.95	1.766
7/15	25.28	33.25	22.1	73.46	97.3	49.61	4.40	7.25	1.554
7/16	23.85	27.71	21.9	82.34	97.3	67.38	4.47	7.25	1.683
7/17	24.35	29.01	22.57	82.79	98.4	67.18	5.39	8.6	2.182
7/18	24.46	29.54	21.65	84.3	97.6	71	6.77	11.9	1.64
7/19	27.68	32.85	23.6	74.56	97.8	51.31	7.88	7.4	2.359

Appendix 1-11 (cont.). Daily average , maximum, and minimum air temperature, % relative humidity, and wind speed during the study period, May 15 – August 17, 2002.

Date	Ave Air Tmp (°C)	Max Air Tmp (°C)	Min Air Tmp (°C)	Ave RH (%)	Max RH (%)	Min RH (%)	Ave WS (mps)	Max WS (mps)	Min WS (mps)
7/20	27.62	32.88	23.53	72.08	97.6	46.55	4.86	7.25	2.464
7/21	27.73	32.96	23.2	70.95	96.5	45.39	7.39	6.5	2.275
7/22	27.81	33.45	22.8	68.45	96.5	40.39	4.78	7.1	2.462
7/23	27.88	33.55	22.97	70.93	97.6	44.25	4.69	6.95	2.433
7/24	27.71	34.38	22.04	69.56	98.4	40.72	4.03	6.5	1.556
7/25	28.59	35.28	22.27	68.68	97.9	39.45	3.84	5.9	1.781
7/26	28.1	34.91	21.04	67.55	96.2	38.89	4.36	6.5	2.222
7/27	28.05	33.95	23.14	70.93	96.9	44.95	5.53	8	3.048
7/28	28.32	33.62	24.5	69.74	93	46.48	6.71	9.5	3.916
7/29	28.67	34.58	24.27	70.33	93.9	46.75	7.53	10.85	4.206
7/30	28.72	34.05	25.23	68.74	91.4	46.08	6.65	9.65	3.648
7/31	28.98	34.55	24.7	66.58	95.3	37.86	6.86	10.25	3.468
8/1	28.65	34.15	24.6	68.28	93.5	43.05	6.01	8.75	3.274
8/2	27.98	34.38	22.37	69.88	96.8	42.95	5.68	8.45	2.903
8/3	28.73	35.15	22.34	67.66	98.5	36.82	3.52	5.6	1.438
8/4	28.31	36.31	20.9	64.65	97.8	31.5	7.75	14	1.49
8/5	27.52	34.71	20.21	67.26	97.2	37.32	3.43	5.45	1.413
8/6	27.77	34.55	21.74	66.28	91.7	40.85	3.84	6.35	1.315
8/7	28.44	35.78	21.6	64.63	94.4	34.86	3.20	5.3	1.099
8/8	29.63	36.31	22.54	61.2	88.6	33.79	4.20	6.8	1.595
8/9	26.54	33.4	23.57	72.7	95.1	50.29	5.12	8.3	1.929
8/10	28.05	34	23.9	69.13	92.9	45.36	5.67	9.8	1.544

Appendix 1-11 (cont.). Daily average , maximum, and minimum air temperature, % relative humidity, and wind speed during the study period, May 15 – August 17, 2002.

Date	Ave Air Tmp (°C)	Max Air Tmp (°C)	Min Air Tmp (°C)	Ave RH (%)	Max RH (%)	Min RH (%)	Ave WS (mps)	Max WS (mps)	Min WS (mps)
8/11	26.75	34.78	22.14	71.81	97.2	46.41	5.09	8.9	1.269
8/12	27.19	34.21	19.85	70.56	98.7	42.42	3.88	6.05	1.714
8/13	28	34.65	21.8	69.48	96.4	42.55	3.84	6.05	1.615
8/14	28.39	35.08	24	66.91	95.2	38.62	6.88	10.4	3.355
8/15	27.61	34.42	23.43	70.66	97.6	43.72	5.65	8.9	2.385
8/16	27.66	34.73	23.77	69.71	91.8	47.62	6.72	9.95	3.494
8/17	28.47	34.75	23.43	68.23	94.6	41.86	6.43	9.8	3.057

Appendix 2-1. *Zizania texana* pollen rain raw data. Study took place May19 – July 3, 2003. Pollen traps were set out at each distance interval (0, 0.75, 1.5, 3, 6, and 12 m) when the number of panicles releasing pollen reached: 1) 4-8 panicles; 2) 9-20 panicles; 3) 21-40 panicles; and 4) 41-100 panicles. Traps remained in place for 24 hours. After 24 hours, the traps were removed and returned to the lab. A cover slip was placed on each slide and pollen grains beneath the cover slip were counted and recorded.

Date	5/19/2003					5/20/2003					5/21/2003					Total Pollen
	Density 1 4 to 8					Density 1 4 to 8					Density 1 4 to 8					
St flwrs blooming	5					7					7					
Raceway	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean	
Distance interval																
0	11	18	4	0	8.25	17	11	67	19	28.5	1	3	0	0	1	151
0.75	9	5	2	0	4	52	9	2	5	17	0	1	1	0	0.5	86
1.5	10	35	1	0	11.5	5	11	7	3	6.5	0	1	2	2	1.25	77
3	3	39	1	0	10.75	17	16	0	8	10.25	0	0	0	1	0.25	85
6	2	38	0	1	10.25	8	14	0	8	7.5	0	0	0	2	0.5	73
12	3	20	0	0	5.75	3	16	0	14	8.25	0	0	0	0	0	56

Appendix 2-1 (continued)

Date	5/28/03					5/29/2003					5/30/2003					Total Pollen
	Density 2 9 to 20					Density 2 9 to 20					Density 2 9 to 20					
St flwrs blooming	9					10					11					
Raceway	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean	
Distance interval																
0	0	4	116	16	34	6	6	97	228	84.25	16	0	51	23	22.5	563
0.75	1	4	75	3	20.75	2	6	145	158	77.75	3	2	19	17	10.25	435
1.5	2	2	47	7	14.5	8	6	64	54	33	1	1	8	14	6	214
3	1	2	14	4	5.25	4	1	33	24	15.5	1	2	17	3	5.75	106
6	2	2	4	6	3.5	5	1	6	6	4.5	1	2	3	6	3	44
12	2	0	3	0	1.25	2	5	3	5	3.75	0	1	0	6	1.75	27

Appendix 2-1 (continued)

Date:	6/9/2003					6/10/2003					6/11/2003					Total Pollen
	Density 3 21 to 40					Density 3 21 to 40					Density 3 21 to 40					
St flwrs blooming	27					26										
Raceway	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean	
Distance interval																
0	2	455	108	78	160.8	44	56	1	2	25.75	82	2	3	3	22.5	836
0.75	2	2	123	8	33.75	15	6	0	5	6.5	29	5	3	3	10	201
1.5	2	0	112	4	29.5	21	10	0	3	8.5	26	3	3	0	8	184
3	0	0	29	1	7.5	9	11	2	0	5.5	1	5	2	2	2.5	62
6	0	1	7	0	2	3	4	1	0	2	2	2	2	1	1.75	23
12	0	1	0	0	0.25	5	7	0	0	3	2	2	2	1	1.75	20

Appendix 2-1 (continued).

Date	7/1/2003					7/2/2003					7/3/2003					Total Pollen
	Density 4 41 to 100					Density 4 41 to 100					Density 4 41 to 100					
St flwrs blooming	67					78					81					
Raceway	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean	
Distance interval																
0	49	506	187	152	224	140	796	115	353	351	74	559	408	433	369	3772
0.75	22	130	152	96	100	161	103	53	76	98	90	90	228	36	111	1237
1.5	13	63	157	54	72	73	77	33	33	54	46	64	102	13	56	718
3	6	5	54	8	18	38	53	10	17	30	22	19	17	8	17	257
6	3	4	10	8	6	18	14	7	7	12	5	8	25	6	11	115
12	6	2	3	8	5	13	15	11	8	12	3	2	5	4	4	80

Appendix 2-2. *Zizania texana* seed set raw data. Study took place June 3 – July 13, 2003. One panicle with receptive stigmas was tagged at each distance interval (0, 0.75, 1.5, 3, 6, and 12 m) when the number of panicles releasing pollen reached: 1) 4-8 panicles; 2) 9-20 panicles; 3) 21-40 panicles; and 4) 41-100 panicles. Panicles were tagged for three consecutive days. When tagged panicles matured to the staminate stage, they were emasculated and each panicle bagged. After 10 days, bagged panicles were clipped and returned to the lab. The number of maturing seeds and pistillate panicles were counted and recorded.

Date: 6/3/03					Date: 6/5/03					Date: 6/6/03				
				Density 1 4 to 8					Density 1 4 to 8					Density 1 4 to 8
Raceway	1	2	3	4	Raceway	1	2	3	4	Raceway	1	2	3	4
Distance interval					Distance interval					Distance interval				
0	4/50				0					0				
0.75					0.75	5/40				0.75				
1.5					1.5					1.5				
3					3					3				
6					6	0/48				6				
12					12	0/29				12				

Appendix 2-2 (continued). *Zizania texana* seed set raw data. Study took place June 3 – July 13, 2003. One panicle with receptive stigmas was tagged at each distance interval (0, 0.75, 1.5, 3, 6, and 12 m) when the number of panicles releasing pollen reached: 1) 4-8 panicles; 2) 9-20 panicles; 3) 21-40 panicles; and 4) 41-100 panicles. Panicles were tagged for three consecutive days. When tagged panicles matured to the staminate stage, they were emasculated and each panicle bagged. After 10 days, bagged panicles were clipped and returned to the lab. The number of maturing seeds and pistillate panicles were counted and recorded.

Date: 6/10/03					Date: 6/12/03					Date: 6/13/03				
Density 2 9 to 20					Density 2 9 to 20					Density 2 9 to 20				
Raceway Distance interval	1	2	3	4	Raceway Distance interval	1	2	3	4	Raceway Distance interval	1	2	3	4
0					0					0				20/83
0.75	0/45				0.75			0/42		0.75				
1.5					1.5				0/66	1.5				
3					3					3				
6					6	0/68				6				
12				0/97	12	0/66				12				

Appendix 2-2 (continued). *Zizania texana* seed set raw data. Study took place June 3 – July 13, 2003. One panicle with receptive stigmas was tagged at each distance interval (0, 0.75, 1.5, 3, 6, and 12 m) when the number of panicles releasing pollen reached: 1) 4-8 panicles; 2) 9-20 panicles; 3) 21-40 panicles; and 4) 41-100 panicles. Panicles were tagged for three consecutive days. When tagged panicles matured to the staminate stage, they were emasculated and each panicle bagged. After 10 days, bagged panicles were clipped and returned to the lab. The number of maturing seeds and pistillate panicles were counted and recorded.

Date: 6/14/03					Date: 6/19/03				
Raceway Distance interval	Density 3 21 to 40				Raceway Distance interval	Density 3 21 to 40			
	1	2	3	4		1	2	3	4
0					0	9/42	3/55	0/57	4/66
0.75				0/70	0.75	8/47		20/77	
1.5	1/85				1.5	2/50		0/104	2/50
3			3/63		3	0/64	0/33	3/82	0/50
6				0/46	6	0/59		0/55	
12					12	0/62		9/65	0/54

Appendix 2-2 (continued). *Zizania texana* seed set raw data. Study took place June 3 – July 13, 2003. One panicle with receptive stigmas was tagged at each distance interval (0, 0.75, 1.5, 3, 6, and 12 m) when the number of panicles releasing pollen reached: 1) 4-8 panicles; 2) 9-20 panicles; 3) 21-40 panicles; and 4) 41-100 panicles. Panicles were tagged for three consecutive days. When tagged panicles matured to the staminate stage, they were emasculated and each panicle bagged. After 10 days, bagged panicles were clipped and returned to the lab. The number of maturing seeds and pistillate panicles were counted and recorded.

Date: 7/11					Date: 7/12/03					Date: 7/13/03				
Density 4					Density 4					Density 4				
41 to 100					41 to 100					41 to 100				
Raceway Distance interval	1	2	3	4	Raceway Distance interval	1	2	3	4	Raceway Distance interval	1	2	3	4
0					0					0				
0.75					0.75					0.75			15/89	
1.5					1.5					1.5			2/110	
3					3					3				
6	0/74				6		0/64			6				
12					12					12	0/80			0/67

Appendix 2-3. Raw data for diel 1 pollen dispersal study, 23 June- 27 June, 2003.

Date: 6/23/03 Time interval: 11p-12a Mean: 13				Date: 6/24/03 Time interval: 5a-6a Mean: 4			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	15	62	77	1	10	3	13
2	3	20	23	2	9	2	11
3	2	0	2	3	2	2	4
4	0	1	1	4	1	3	4

Date: 6/24/03 Time interval: 12a-1a Mean: 18				Date: 6/24/03 Time interval: 6a-7a Mean: 17			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	58	23	81	1	26	23	49
2	4	57	61	2	40	26	66
3	1	0	1	3	3	5	8
4	3	0	3	4	7	4	11

Date: 6/24/03 Time interval: 1a-2a Mean: 16				Date: 6/24/03 Time interval: 7a-8a Mean: 9.25			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	25	38	63	1	33	9	42
2	9	30	39	2	21	5	26
3	5	13	18	3	0	1	1
4	5	5	10	4	3	2	5

**Appendix 2-3 (continued). Raw data for diel 1 pollen dispersal study,
23 June- 27 June, 2003.**

Date: 6/24/03 Time interval: 2a-3a Mean: 47				Date: 6/24/03 Time interval: 8a-9a Mean: 25			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	188	99	287	1	38	58	96
2	65	4	69	2	101	0	101
3	6	4	10	3	0	1	1
4	6	6	12	4	1	3	4
Date: 6/24/03 Time interval: 3a-4a Mean: 9				Date: 6/24/03 Time interval: 9a-10a Mean: 7.5			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	21	32	53	1	31	11	42
2	2	1	3	2	6	6	12
3	1	3	4	3	1	0	1
4	7	5	12	4	2	3	5
Date: 6/24/03 Time interval: 4a-5a Mean: 8.75				Date: 6/24/03 Time interval: 10a-11a Mean: 8.875			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	37	12	49	1	24	10	34
2	12	0	12	2	19	12	31
3	2	2	4	3	2	0	2
4	2	3	5	4	0	4	4

**Appendix 2-3 (continued). Raw data for diel 1 pollen dispersal study,
23 June- 27 June, 2003.**

Date: 6/24/03				Date: 6/25/03			
Time interval: 11p-12a				Time interval: 5a-6a			
Mean: 11				Mean: 27.6			
	Pollen count				Pollen count		
Raceway	A	B	Total	Raceway	A	B	Total
1	11	32	43	1	35	42	77
2	41	1	42	2	5	131	136
3	0	0	0	3	4	1	5
4	1	3	4	4	2	1	3
Date: 6/25/03				Date: 6/25/03			
Time interval: 12a-1a				Time interval: 6a-7a			
Mean: 26				Mean: 32.2			
	Pollen count				Pollen count		
Raceway	A	B	Total	Raceway	A	B	Total
1	11	35	46	1	55	39	94
2	155	2	157	2	89	44	133
3	1	2	3	3	5	7	12
4	2	0	2	4	5	14	19
Date: 6/25/03				Date: 6/25/03			
Time interval: 1a-2a				Time interval: 7a-8a			
Mean: 19				Mean: 21.6			
	Pollen count				Pollen count		
Raceway	A	B	Total	Raceway	A	B	Total
1	30	14	44	1	44	34	78
2	2	85	87	2	12	71	83
3	1	9	10	3	2	1	3
4	3	7	10	4	5	4	9

**Appendix 2-3 (continued). Raw data for diel 1 pollen dispersal study,
23 June- 27 June, 2003.**

Date: 6/25/03 Time interval: 2a-3a Mean: 43				Date: 6/25/03 Time interval: 8a-9a Mean: 26			
Raceway	Pollen count			Raceway	Pollen count		
	A	B	Total		A	B	Total
1	32	48	80	1	19	134	153
2	10	206	216	2	9	6	15
3	15	6	21	3	26	4	30
4	8	15	23	4	6	4	10
Date: 6/25/03 Time interval: 3a-4a Mean: 33				Date: 6/25/03 Time interval: 9a-10a Mean: 1.25			
Raceway	Pollen count			Raceway	Pollen count		
	A	B	Total		A	B	Total
1	61	37	98	1	0	1	1
2	145	3	148	2	1	5	6
3	8	3	11	3	1	2	3
4	2	5	7	4	0	0	0
Date: 6/25/03 Time interval: 4a-5a Mean: 43.9				Date: 6/25/03 Time interval: 10a-11a Mean: 1.6			
Raceway	Pollen count			Raceway	Pollen count		
	A	B	Total		A	B	Total
1	69	71	140	1	3	0	3
2	155	19	174	2	3	2	5
3	12	14	26	3	0	1	1
4	6	5	11	4	2	2	4

**Appendix 2-3 (continued). Raw data for diel 1 pollen dispersal study,
23 June- 27 June, 2003.**

Date: 6/25/03 Time interval: 11p-12a Mean: 2				Date: 6/26/03 Time interval: 5a-6a Mean: 7.4			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	2	5	7	1	3	41	44
2	5	5	10	2	15	0	15
3	0	0	0	3	0	0	0
4	0	0	0	4	0	0	0
Date: 6/26/03 Time interval: 12a-1a Mean: 2				Date: 6/26/03 Time interval: 6a-7a Mean: 18			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	3	1	4	1	53	61	114
2	6	2	8	2	10	10	20
3	0	0	0	3	6	5	11
4	0	1	1	4	1	0	1
Date: 6/26/03 Time interval: 1a-2a Mean: 10				Date: 6/26/03 Time interval: 7a-8a Mean: 21.7			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	10	11	21	1	26	60	86
2	26	7	33	2	55	31	86
3	1	1	2	3	2	0	2
4	16	4	20	4	0	0	0

**Appendix 2-3 (continued). Raw data for diel 1 pollen dispersal study,
23 June- 27 June, 2003.**

Date: 6/26/03 Time interval: 2a-3a Mean: 31				Date: 6/26/03 Time interval: 8a-9a Mean: 29.4			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	78	59	137	1	78	57	135
2	59	1	60	2	12	39	51
3	4	3	7	3	11	2	13
4	14	26	40	4	15	21	36
Date: 6/26/03 Time interval: 3a-4a Mean: 52				Date: 6/26/03 Time interval: 9a-10a Mean: 7.5			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	63	95	158	1	9	22	31
2	151	16	167	2	19	2	21
3	4	13	17	3	2	4	6
4	29	43	72	4	1	1	2
Date: 6/26/03 Time interval: 4a-5a Mean: 28.5				Date: 6/26/03 Time interval: 10a-11a Mean: 6.2			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	52	27	79	1	37	1	38
2	80	9	89	2	6	0	6
3	11	17	28	3	0	0	0
4	14	18	32	4	1	5	6

**Appendix 2-3 (continued). Raw data for diel 1 pollen dispersal study,
23 June- 27 June, 2003.**

Date: 6/26/03 Time interval: 11p-12a Mean: 12.6				Date: 6/27/03 Time interval: 5a-6a Mean: 14			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	1	0	1	1	1	1	2
2	6	1	7	2	2	3	5
3	24	67	91	3	58	27	85
4	1	1	2	4	2	19	21
Date: 6/27/03 Time interval: 12a-1a Mean: 11.6				Date: 6/27/03 Time interval: 6a-7a Mean: 9.2			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	2	1	3	1	2	5	7
2	25	3	28	2	2	9	11
3	48	11	59	3	17	34	51
4	0	3	3	4	2	3	5
Date: 6/27/03 Time interval: 1a-2a Mean: 28				Date: 6/27/03 Time interval: 7a-8a Mean: 8.7			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	0	2	2	1	1	3	4
2	0	40	40	2	6	3	9
3	3	168	171	3	37	19	56
4	1	10	11	4	1	0	1

**Appendix 2-3 (continued). Raw data for diel 1 pollen dispersal study,
23 June- 27 June, 2003.**

Date: 6/27/03 Time interval: 2a-3a Mean: 49				Date: 6/27/03 Time interval: 8a-9a Mean: 43			
Raceway	Pollen count			Raceway	Pollen count		
	A	B	Total		A	B	Total
1	0	2	2	1	0	1	1
2	1	69	70	2	93	4	97
3	50	234	284	3	153	53	206
4	34	1	35	4	0	44	44
Date: 6/27/03 Time interval: 3a-4a Mean: 37.25				Date: 6/27/03 Time interval: 9a-10a Mean: 6			
Raceway	Pollen count			Raceway	Pollen count		
	A	B	Total		A	B	Total
1	0	1	1	1	0	2	2
2	1	22	23	2	3	10	13
3	225	38	263	3	30	4	34
4	0	11	11	4	0	1	1
Date: 6/27/03 Time interval: 4a-5a Mean: 28.6				Date: 6/27/03 Time interval: 10a-11a Mean: 5			
Raceway	Pollen count			Raceway	Pollen count		
	A	B	Total		A	B	Total
1	1	0	1	1	1	0	1
2	16	7	23	2	8	0	8
3	154	40	194	3	4	5	9
4	1	12	13	4	2	1	3

Appendix 2-4. Raw data for diel 2 pollen dispersal study, 21 July- 24 July, 2003.

Date: 7/21/03 Time interval: 11a-12p Mean: 0				Date: 7/21/03 Time interval: 5p-6p Mean: 1			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	0	0	0	1	0	2	2
2	0	0	0	2	3	3	6
3	0	0	0	3	0	0	0
4	0	0	0	4	0	0	0
Date: 7/21/03 Time interval: 12p-1p Mean: 0				Date: 7/21/03 Time interval: 6p-7p Mean: 0.125			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	0	0	0	1	0	0	0
2	0	0	0	2	0	0	0
3	0	0	0	3	1	0	1
4	0	0	0	4	0	0	0
Date: 7/21/03 Time interval: 1p-2p Mean: 2				Date: 7/21/03 Time interval: 7p-8p Mean: 0			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	4	2	6	1	0	0	0
2	6	0	6	2	0	0	0
3	0	2	2	3	0	0	0
4	2	0	2	4	0	0	0

**Appendix 2-4 (continued). Raw data for diel 2 pollen dispersal study,
21 July- 24 July, 2003.**

Date: 7/21/03 Time interval: 2p-3p Mean: 1				Date: 7/21/03 Time interval: 8p-9p Mean: 0.5			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	2	4	6	1	0	1	1
2	0	1	1	2	0	3	3
3	2	2	2	3	0	0	0
4	1	1	2	4	0	0	0
Date: 7/21/03 Time interval: 3p-4p Mean: 6				Date: 7/21/03 Time interval: 9p-10p Mean: 0.25			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	3	0	3	1	1	0	1
2	3	31	34	2	1	0	1
3	1	0	1	3	0	0	0
4	0	6	6	4	0	0	0
Date: 7/21/03 Time interval: 4p-5p Mean: 0.5				Date: 7/21/03 Time interval: 10p-11p Mean: 0.125			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	1	0	1	1	0	0	0
2	0	0	0	2	0	0	0
3	2	1	3	3	0	0	0
4	0	0	0	4	1	0	1

**Appendix 2-4 (continued). Raw data for diel 2 pollen dispersal study,
21 July- 24 July, 2003.**

Date: 7/22/03 Time interval: 11a-12p Mean: 0.625				Date: 7/22/03 Time interval: 5p-6p Mean: 0			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	1	1	2	1	0	0	0
2	0	1	1	2	0	0	0
3	1	0	1	3	0	0	0
4	0	1	1	4	0	0	0
Date: 7/22/03 Time interval: 12p-1p Mean: 1.6				Date: 7/22/03 Time interval: 6p-7p Mean: 0.125			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	6	0	6	1	0	0	0
2	4	1	5	2	0	0	0
3	2	0	2	3	0	0	0
4	0	0	0	4	0	1	1
Date: 7/22/03 Time interval: 1p-2p Mean: 0.5				Date: 7/22/03 Time interval: 7p-8p Mean: 0.25			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	1	1	2	1	0	2	2
2	0	0	0	2	0	0	0
3	0	1	1	3	0	0	0
4	1	0	1	4	0	0	0

**Appendix 2-4 (continued). Raw data for diel 2 pollen dispersal study,
21 July- 24 July, 2003.**

Date: 7/22/03 Time interval: 2p-3p Mean: 0.75				Date: 7/22/03 Time interval: 8p-9p Mean: 0.625			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	1	2	3	1	0	0	0
2	0	0	0	2	0	0	0
3	0	2	2	3	0	4	4
4	0	1	1	4	0	1	1
Date: 7/22/03 Time interval: 3p-4p Mean: 0.25				Date: 7/22/03 Time interval: 9p-10p Mean: 0.5			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	0	0	0	1	0	0	0
2	1	0	1	2	0	0	0
3	0	0	0	3	2	1	3
4	1	0	1	4	0	1	1
Date: 7/22/03 Time interval: 4p-5p Mean: 0				Date: 7/22/03 Time interval: 10p-11p Mean: 0.375			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	0	0	0	1	0	0	0
2	0	0	0	2	1	2	3
3	0	0	0	3	0	0	0
4	0	0	0	4	0	0	0

**Appendix 2-4 (continued). Raw data for diel 2 pollen dispersal study,
21 July- 24 July, 2003.**

Date: 7/23/03 Time interval: 11a-12p Mean: 0.625				Date: 7/23/03 Time interval: 5p-6p Mean: 0.125			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	1	0	1	1	0	0	0
2	2	1	3	2	0	0	0
3	1	0	1	3	0	0	0
4	0	0	0	4	1	0	1
Date: 7/23/03 Time interval: 12p-1p Mean: 0.125				Date: 7/23/03 Time interval: 6p-7p Mean: 0.125			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	1	0	1	1	0	0	0
2	0	0	0	2	0	0	0
3	0	0	0	3	1	0	1
4	0	0	0	4	0	0	0
Date: 7/23/03 Time interval: 1p-2p Mean: 1.75				Date: 7/23/03 Time interval: 7p-8p Mean: 0			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	0	5	5	1	0	0	0
2	2	1	3	2	0	0	0
3	3	1	4	3	0	0	0
4	0	2	0	4	0	0	0

**Appendix 2-4 (continued). Raw data for diel 2 pollen dispersal study,
21 July- 24 July, 2003.**

Date: 7/23/03 Time interval: 2p-3p Mean: 0.75				Date: 7/23/03 Time interval: 8p-9p Mean: 0.125			
	Pollen count				Pollen count		
Raceway	A	B	Total	Raceway	A	B	Total
1	1	1	2	1	0	0	0
2	0	0	0	2	1	0	1
3	1	1	2	3	0	0	0
4	0	2	2	4	0	0	0
Date: 7/23/03 Time interval: 3p-4p Mean:				Date: 7/23/03 Time interval: 9p-10p Mean: 0.375			
	Pollen count				Pollen count		
Raceway	A	B	Total	Raceway	A	B	Total
1	RAINED OUT			1	2	0	2
2			0	0	0		
3			1	0	1		
4			0	0	0		
Date: 7/23/03 Time interval: 4p-5p Mean: 0.25				Date: 7/23/03 Time interval: 10p-11p Mean: 0			
	Pollen count				Pollen count		
Raceway	A	B	Total	Raceway	A	B	Total
1	0	1	1	1	0	0	0
2	0	0	0	2	0	0	0
3	1	0	1	3	0	0	0
4	0	0	0	4	0	0	0

**Appendix 2-4 (continued). Raw data for diel 2 pollen dispersal study,
21 July- 24 July, 2003.**

Date: 7/24/03 Time interval: 11a-12p Mean: 0.625				Date: 7/24/03 Time interval: 5p-6p Mean: 0.625			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	2	0	2	1	0	0	0
2	0	0	0	2	0	0	0
3	0	0	0	3	0	0	0
4	1	2	3	4	1	4	5
Date: 7/24/03 Time interval: 12p-1p Mean: 0.375				Date: 7/24/03 Time interval: 6p-7p Mean: 0.125			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	1	0	1	1	0	1	1
2	0	0	0	2	0	0	0
3	0	0	0	3	0	0	0
4	0	2	2	4	0	0	0
Date: 7/24/03 Time interval: 1p-2p Mean: 0.875				Date: 7/24/03 Time interval: 7p-8p Mean: 1.125			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	2	1	3	1	1	0	1
2	1	0	1	2	0	1	1
3	1	0	1	3	3	0	3
4	2	0	2	4	1	3	4

**Appendix 2-4 (continued). Raw data for diel 2 pollen dispersal study,
21 July- 24 July, 2003.**

Date: 7/24/03 Time interval: 2p-3p Mean: 0.5				Date: 7/24/03 Time interval: 8p-9p Mean: 5.0			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	0	2	2	1	15	8	23
2	1	0	1	2	9	4	13
3	0	0	0	3	0	0	0
4	0	1	1	4	2	1	3
Date: 7/24/03 Time interval: 3p-4p Mean: 0.5				Date: 7/24/03 Time interval: 9p-10p Mean: 0.25			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	1	0	1	1	1	0	1
2	1	1	2	2	0	0	0
3	1	0	1	3	1	0	1
4	0	0	0	4	0	0	0
Date: 7/24/03 Time interval: 4p-5p Mean: 0.375				Date: 7/24/03 Time interval: 10p-11p Mean: 2.25			
Raceway	Pollen count		Total	Raceway	Pollen count		Total
	A	B			A	B	
1	0	2	2	1	13	2	15
2	0	0	0	2	0	0	0
3	0	0	0	3	0	1	1
4	1	0	1	4	2	0	2

Appendix 2-5. Raw data for six hour pollen dispersal study, 16 June - 18 June, 2003.

Date: 6/16/03

Time interval: 5 am - 11 am

Staminate flowers: 30

Mean: 269

	Pollen count		Total
Raceway			
1	7	7	14
2	14	6	20
3	847	43	890
4	941	286	1227

Date: 6/16/03

Time interval: 11 am - 5 pm

Staminate flowers:

Mean: 19

	Pollen count		Total
Raceway			
1	0	1	1
2	28	0	28
3	8	28	36
4	5	79	84

Date: 6/16/03

Time interval: 5 pm - 11 pm

Staminate flowers: 40

Mean: 4

	Pollen count		Total
Raceway			
1	1	5	6
2	1	1	2
3	2	1	3
4	14	7	21

**Appendix 2-5 (continued). Raw data for six hour pollen dispersal study,
16 June - 18 June, 2003.**

Date: 6/16/03

Time interval: 11 pm - 5 am

Staminate flowers: 40

Mean: 305

	Pollen count		Total
Raceway			
1	1	4	5
2	9	15	24
3	188	159	347
4	1413	652	2065

Date: 6/17/03

Time interval: 5 am - 11 am

Staminate flowers: 44 (emasculated 4)

Mean: 274

	Pollen count		Total
Raceway			
1	20	17	37
2	7	19	25
3	37	676	713
4	652	765	1417

Date: 6/17/03

Time interval: 11 am - 5 pm

Staminate flowers: 48 (emasculated 8)

Mean: 9

	Pollen count		Total
Raceway			
1	1	2	3
2	1	0	1
3	34	14	48
4	11	8	19

**Appendix 2-5 (continued). Raw data for six hour pollen dispersal study,
16 June - 18 June, 2003.**

Date: 6/17/03

Time interval: 5 pm - 11 pm

Staminate flowers: 42 (emasculated 2)

Mean: 2.3

	Pollen count		Total
Raceway			
1	1	2	3
2	0	0	0
3	3	2	5
4	7	4	11

Date: 6/17/03

Time interval: 11 pm - 5 am

Staminate flowers: 42 (emasculated 2)

Mean: 168

	Pollen count		Total
Raceway			
1	0	0	0
2	10	0	10
3	458	310	768
4	312	255	567

Date: 6/18/03

Time interval: 5 am - 11 am

Staminate flowers: 34

Mean: 223

	Pollen count		Total
Raceway			
1	4	2	6
2	2	2	4
3	48	246	294
4	872	610	1482

**Appendix 2-5 (continued). Raw data for six hour pollen dispersal study,
16 June - 18 June, 2003.**

Date: 6/18/03

Time interval: 11 am - 5 pm

Staminate flowers: 48 (emasculated 8)

Mean: 2.88

	Pollen count		Total
Raceway			
1	3	1	4
2	3	3	6
3	5	5	10
4	1	2	3

Date: 6/18/03

Time interval: 5 pm - 11 pm

Staminate flowers: 39

Mean: 3.8

	Pollen count		Total
Raceway			
1	0	0	0
2	1	3	4
3	15	4	19
4	5	3	8

Date: 6/18/03

Time interval: 11 pm - 5 am

Staminate flowers:

Mean: 86

	Pollen count		Total
Raceway			
1	16	4	20
2	16	7	23
3	138	322	460
4	144	42	186

Appendix 2-6. Weather conditions throughout the entire study period, 19 May - 24 July, 2003.

Date	P mb	Air Tmp C AVG	Air Tmp C MAX	Air Tmp C MIN	RH %MAX	RH %MIN	kW pm2	WS mps MAX	WS mps MIN	Rain mm TOT
139	998	20.56	26.94	15.59	73.2	22.48	0.312	12.5	5.095	0
140	997	18.35	26.41	11.64	75.6	17.89	0.339	7.25	2.403	0
141	997	18.9	27.44	9.94	75.4	25.91	0.296	6.8	2.3	0
142	995	20.96	28.84	12.07	94.2	33.87	0.3	9.8	3.051	0
143	992	22.73	31.05	13.66	97.4	33.79	0.257	11.15	3.736	0
144	991	24.79	30.99	20.54	89.5	39.02	0.253	11.75	4.602	0
145	990	23.54	27.68	20.1	91.7	58.88	0.106	8.3	2.983	0
146	992	25.75	32.48	20.14	94.2	38.89	0.247	6.65	2.154	0
147	991	26.16	32.88	19.6	92.6	37.96	0.281	12.8	2.821	6.604
148	989	25.67	32.82	19.1	97.9	34.89	0.289	10.1	3.198	0
149	988	24.08	30.07	16.65	98.6	48.52	0.243	11	2.522	39.37
150	986	23.94	32.08	18.12	97.3	51.17	0.238	12.05	2.192	21.59
151	986	26.64	35.91	18.84	98.6	22.37	0.29	8.3	1.836	0
152	988	28.01	31.85	22.5	72.9	38.39	0.327	8	2.999	0
153	988	25.51	31.82	18.11	93.9	40.52	0.309	5.75	1.779	0
154	987	25.77	31.98	19.27	96	45.31	0.301	6.95	2.179	0
155	987	26.71	33.21	21.7	97.4	43.75	0.252	8.6	2.506	0
156	988	27.88	34.01	23.7	93.1	37.99	0.261	9.65	3.382	0
157	990	28.19	34.18	23.2	92.7	32.56	0.283	8	2.146	0
158	990	28.42	34.05	22.64	80.5	35.49	0.308	7.1	2.765	0
159	989	27.69	34.08	21.27	88.7	42.12	0.295	8.45	1.836	0
160	989	27.85	34.61	22.57	95.5	34.19	0.279	8.3	2.318	0
161	987	27.97	33.39	24.23	92.9	46.98	0.223	11.45	3.429	0
162	988	28.82	35.21	24.62	89.8	37.42	0.256	8.75	3.652	0
163	988	28.9	35.74	23.87	94.4	33.26	0.268	7.7	2.883	0
164	988	28.8	35.94	23.5	95.7	37.06	0.281	8.9	2.531	0
165	988	28.5	34.81	22.87	94	34.86	0.31	7.55	1.927	0
166	991	28.96	35.78	21.47	95.5	37.39	0.24	7.55	2.191	0
167	987	27.74	34.18	22.24	88.6	37.33	0.25	7.1	2.325	0
168	990	25.61	32.55	18.6	97.3	41.92	0.283	14.15	3.458	24.89
169	988	25.65	32.78	19.05	91.1	31.06	0.326	6.2	1.835	0

Appendix 2-6 (continued). Weather conditions throughout the entire study period, 19 May - 24 July, 2003.

Date	P mb	Air Tmp C AVG	Air Tmp C MAX	Air Tmp C MIN	RH %MAX	RH %MIN	kW pm2	WS mps MAX	WS mps MIN	Rain mm TOT
170	989	26	33.18	17.66	93	31.63	0.319	7.25	2.103	0
171	993	27.44	35.11	18.84	97.6	34.36	0.295	7.85	2.266	0
172	997	28.01	33.91	23.47	96.2	37.36	0.28	10.4	2.764	0
173	997	28.04	34.45	21.1	97	31.03	0.316	7.25	1.686	0
174	995	27.94	34.68	20.7	96	29.17	0.315	6.65	1.698	0
175	992	27.27	34.65	17.92	93.6	25.5	0.331	7.25	1.409	0
176	992	27.8	34.95	19.61	87.4	28.73	0.318	8.45	1.872	0
177	992	27.33	36.58	19.9	95	30.03	0.302	13.25	1.802	0
178	990	26.7	36.44	18.54	98	33.83	0.268	13.4	1.945	12.7
179	989	25.36	32.09	18.91	97.2	44.55	0.241	7.1	2.194	0
180	991	25.5	33.48	22.07	94.9	46.05	0.218	12.65	1.616	0
181	993	23.77	27.01	22.17	97.7	70	0.093	6.8	2.488	12.45
182	994	23.32	29.14	20.47	98.9	68.11	0.095	14.9	2.702	109.7
183	993	23.1	24.6	20.84	98.4	83.2	0.041	7.25	1.913	48.77
184	992	23.21	24.43	19.14	99.2	85.5	0.049	9.8	2.406	54.86
185	990	26.3	32.23	21.1	97.9	47.05	0.221	11.6	3.506	4.064
186	992	26.85	32.32	23.53	90.7	46.19	0.21	10.4	3.347	4.318
187	993	25.54	30.61	21.67	97.5	65.71	0.132	12.05	2.344	23.37
188	993	26.73	32.88	21.1	98.9	51.91	0.216	6.2	1.378	0
189	995	26.43	33.35	21.57	98.6	48.18	0.211	10.4	1.182	13.21
190	995	27.95	34.88	22.14	96.5	41.32	0.28	10.4	1.761	0
191	995	26.39	33.15	22.67	96.2	48.21	0.266	11.9	1.878	5.588
192	995	26.95	33.31	22.67	97.7	41.68	0.309	6.2	1.377	0
193	991	27.35	34.21	22.2	98.1	45.95	0.246	6.65	1.207	0
194	988	28.67	35.68	22.27	89.3	37.52	0.274	5	1.561	0
195	990	24.84	29.41	22.41	95.2	67.14	0.075	6.95	1.766	6.604
196	991	25.28	33.25	22.1	97.3	49.61	0.186	7.25	1.554	0
197	993	23.85	27.71	21.9	97.3	67.38	0.12	7.25	1.683	1.016
198	994	24.35	29.01	22.57	98.4	67.18	0.126	8.6	2.182	10.67
199	995	24.46	29.54	21.65	97.6	71	0.14	11.9	1.64	6.35
200	993	27.68	32.85	23.6	97.8	51.31	0.291	7.4	2.359	0
201	993	27.62	32.88	23.53	97.6	46.55	0.293	7.25	2.464	0

Appendix 2-6 (continued). Weather conditions throughout the entire study period, 19 May - 24 July, 2003.

Date	P mb	Air Tmp C AVG	Air Tmp C MAX	Air Tmp C MIN	RH %MAX	RH %MIN	kW pm2	WS mps MAX	WS mps MIN	Rain mm TOT
200	993	27.68	32.85	23.6	97.8	51.31	0.291	7.4	2.359	0
201	993	27.62	32.88	23.53	97.6	46.55	0.293	7.25	2.464	0
202	994	27.73	32.96	23.2	96.5	45.39	0.28	6.5	2.275	0
203	994	27.81	33.45	22.8	96.5	40.39	0.292	7.1	2.462	0
204	996	27.88	33.55	22.97	97.6	44.25	0.279	6.95	2.433	0
205	997	27.71	34.38	22.04	98.4	40.72	0.285	6.5	1.556	0

Appendix 3-1. Raw data for seasonal variation in *Z. texana* seed production.

Date	Sink wt	Sink no	Sink ave wt	Float wt	Float no	Float ave wt	Total wt	Total no	Total ave wt	No inflor	Seeds/inflor
5/14/03	0.314	26	0.12	0.22	2	0.011	0.336	28	0.012		
5/19/03	0.077	6	0.013	0	0		0.077	6	0.013		
5/29/03	0.044	4	0.011	0.053	5	0.011	0.097	9	0.011		
6/2/03	0.035	3	0.012	0.156	14	0.011	0.191	17	0.011		
6/5/03	0.21	16	0.013	0.183	16	0.011	0.393	32	0.012		
6/9/03	0.012	1	0.012	0.168	18	0.009	0.18	19	0.009		
6/13/03	0.169	15	0.011	0.521	51	0.010	0.69	66	0.010		
6/15/03	0.365	31	0.012	0.275	24	0.011	0.64	55	0.012		
6/19/03	0.381	30	0.013	0.416	37	0.011	0.797	67	0.012		
6/24/03	0.702	54	0.013	0.557	47	0.012	1.259	101	0.012		
6/30/03	1.142	84	0.014	0.813	76	0.011	1.955	160	0.012		
7/3/03	3.341						3.341				
7/7/03	2.564						2.564				
7/11/03	1.339						1.339				
7/14/03	1.196						1.196				
7/22/03	0.786						0.786				
7/24/03	0.929						0.929				
8/8/03	1.977						1.977				
8/10/03	1.719						1.719				
8/27/03	0.616						0.616				
9/17/03	3.77						3.77				
9/30/03	2.11						2.11				
10/6/03	3.13						3.13				
6/10/02							0.28	23	0.012		
6/14/02							0.012	2	0.006		
6/14/02							0.18	14	0.013		
6/14/02							0.18	13	0.014		
6/14/02							0.36	27	0.013		

Appendix 3-1 (continued). Raw data for seasonal variation in *Z. texana* seed production.

Date	Sink wt	Sink no	Sink ave wt	Float wt	Float no	Float ave wt	Total wt	Total no	Total ave wt	No inflor	Seeds/inflor
6/18/02							0.032	3	0.011		
6/18/02							0.044	4	0.011		
6/18/02							0.69	67	0.010		
6/20/02							0.08	7	0.011		
6/20/02							0.48	52	0.009		
6/20/02							0.45	52	0.009		
6/27/02							0.014	2	0.007		
6/27/02							0.2	22	0.009		
6/27/02							0.6	60	0.010		
7/2/02							0.49	52	0.009		
7/8/02							0.03	5	0.006		
7/8/02							0.98	113	0.009		
7/11/02							0.03	4	0.008		
7/11/02							0.1	13	0.008		
7/11/02							1.92	192	0.010		
7/16/02							0.006	1	0.006		
7/16/02							1.39	146	0.010		
7/19/02							0.04	9	0.004		
7/19/02							0.24	33	0.007		
7/23/02							3.23				
7/25/02							2.56	246	0.010		
7/29/02							2.23	205	0.011		
7/31/02							2.42	226	0.011		
8/5/02				1.33	138	0.010	1.33	138	0.010		
8/6/02							0.33	38	0.009		
8/8/02							0.0086	7	0.001		
8/8/02							2.37				
8/13/02							0.0098	1	0.010		

Appendix 3-1 (continued). Raw data for seasonal variation in *Z. texana* seed production.

Date	Sink wt	Sink no	Sink ave wt	Float wt	Float no	Float ave wt	Total wt	Total no	Total ave wt	No inflor	Seeds/inflor
8/13/02							0.026	3	0.009		
8/13/02							2.74	317	0.009		
8/16/02							1.28	138	0.009		
8/20/02							2.05	264	0.008		
8/21/02	0.55	58	0.009	3.39	334	0.010	3.94	392	0.010		
8/26/02							0.054	7	0.008		
8/26/02							1.44	124	0.012		
8/26/02	0.17	15	0.011				0.17	15	0.011		
8/30/02				1.27	133	0.010	1.27	133	0.010		
9/4/02				0.85	85	0.010	0.85	85	0.010		
9/6/02							0.04	15	0.003		
9/6/02							1.24	172	0.007		
9/9/02							1.41	174	0.008		
11/9/02							1.35				
9/16/02							0.008	1	0.008		
9/16/02							0.02	3	0.007		
9/16/02							4.06				
9/23/02							0.05	7	0.007		
9/23/02							0.64				
9/27/02							0.03	4	0.008		
9/27/02							1.67				
9/30/02	1.21	94	0.013	1.82	181	0.010	3.03	275	0.011		
10/4/02							4.53				
10/4/02	0.54	39	0.014	0.66	57	0.012	1.2	96	0.13		
10/9/02							2.5				
10/16/02	2	148	0.014	2.52	227	0.011	4.52	375	0.012		
10/18/02	0.7	58	0.012	2.98	281	0.011	3.68	339	0.011		
10/21/02	1.09	84	0.13	3.05	268	0.011	4.14	352	0.012		

Appendix 3-1 (continued). Raw data for seasonal variation in *Z. texana* seed production.

Date	Sink wt	Sink no	Sink ave wt	Float wt	Float no	Float ave wt	Total wt	Total no	Total ave wt	No inflor	Seeds/inflor
10/30/02	1.53	102	0.015	2.87	249	0.012	4.4	351	0.013		
11/1/02	0.1	8	0.013	1.12	114	0.010	1.22	122	0.010		
11/6/02	0.19	11	0.017	0.74	55	0.013	0.93	66	0.014		
11/8/02	0.1	5	0.020	1.05	88	0.012	1.15	93	0.012		
11/11/02	0.32	20	0.016	1.91	144	0.013	2.23	164	0.014		
11/13/02	0.07	4	0.018	0.73	64	0.011	0.8	68	0.012		
1/3/01	0.18	20	0.009	3.35	416	0.008	3.53	436	0.008		
1/11/01	0			1.53	177	0.009	1.53	177	0.009		
1/25/01	0			0.7	106	0.007	0.7	106	0.007		
2/1/01	0			0.7	88	0.008	0.7	88	0.008		
2/8/01	0			0.42	47	0.009	0.42	47	0.009		
3/15/01	0.06	4	0.015	0.27	33	0.008	0.33	37	0.009		
3/29/01							0.33	37	0.009		
4/5/01							0.52	52	0.010		
4/19/01	14.28	870	0.016	0.17	15	0.011	14.45	885	0.016		
4/26/01	7.29	438	0.017	0.81	58	0.014	8.1	496	0.016		
5/3/01	19.89	1260	0.016	2.19	188	0.012	22.08	1448	0.015		
5/8/01							13.01				
5/15/01							15.94				
5/17/01	4.13	288	0.014	1.06	97	0.011	5.19	385	0.013		
5/24/01	6.27	356	0.018	1.15	83	0.014	7.42	439	0.017		
5/31/01	7.75	493	0.016	6.46	481	0.013	14.21	974	0.015		
6/4/01							3.45				
6/8/01	7.31	494	0.015	3.74	316	0.012	11.05	810	0.014		
6/21/01	2.27	174	0.013	1.45	149	0.010	3.72	323	.0012		
6/26/01							288				
6/28/01	3.99	262	0.015	3.41	286	0.012	7.4	548	0.014		
7/1/01							1.78				

Appendix 3-1 (continued). Raw data for seasonal variation in *Z. texana* seed production.

Date	Sink wt	Sink no	Sink ave wt	Float wt	Float no	Float ave wt	Total wt	Total no	Total ave wt	No inflor	Seeds/inflor
7/6/01	1.87	143	0.013	4.51	495	0.009	6.38	638	0.010		
7/9/01							6.1				
7/11/01	3.51	236	0.015	5.01	321	0.016	8.52	557	0.015		
7/12/01							3.54				
7/18/01							3.33				
7/20/01	2.76	211	0.013	4.98	454	0.011	7.74	665	0.012		
7/24/01							3.37				
7/30/01							3.32				
8/2/01	0.32	33	0.010	1.03	126	0.008	1.35	159	0.008		
8/9/01	0.68	48	0.014	1.18	150	0.008	1.86	198	0.009		
8/16/01	0.77	68	0.011	1.64	176	0.009	2.41	244	0.010		
9/25/01							1.24				
10/2/01							2.81				
10/9/01							3.91				
10/22/01							8.55				
11/8/01							3.98				
11/13/01							1.05				
1/3/00	0.17	13	0.013	0.33	31	0.011	0.5	44	0.011		
2/8/00	0	0		0.05	5	0.010	0.05	5	0.010		
4/24/00	0.34	25	0.014	0.35	34	0.010	0.69	59	0.012		
5/2/00	0.45	32	0.014	0.08	9	0.009	0.53	41	0.013	8	5.1
5/9/00	2.67	168	0.016	0.53	35	0.015	3.2	203	0.016	22	9.2
5/16/00	1.98	133	0.015	0.66	50	0.013	2.64	183	0.014	24	7.6
5/23/00	2.51	153	0.016	0.26	16	0.016	2.77	169	0.016	13	13.0
5/30/00	4.65	290	0.016	1.74	162	0.011	6.39	452	0.014	27	16.7
6/5/00	4.39	329	0.013	2.33	233	0.010	6.72	562	0.012	34	16.5
6/14/00	2.64	151	0.017	1.38	109	0.013	4.02	260	0.015	22	11.8
6/20/00	1.8	128	0.014	1.46	159	0.009	3.26	287	0.011	26	11.0

Appendix 3-1 (continued). Raw data for seasonal variation in *Z. texana* seed production.

Date	Sink wt	Sink no	Sink ave wt	Float wt	Float no	Float ave wt	Total wt	Total no	Total ave wt	No inflor	Seeds/inflor
6/27/00	1.8	114	0.016	1.4	140	0.010	3.2	254	0.013	14	18.1
7/5/00	2.04	143	0.014	2.09	206	0.010	4.13	349	0.012	27	12.9
7/11/00	1	88	0.011	0.56	66	0.008	1.56	154	0.010	16	9.6
7/18/00	1.56	87	0.018	3.22	257	0.013	4.78	344	0.014	29	11.9
7/26/00	0.61	58	0.011	0.98	125	0.008	1.59	183	0.009	23	8.0
8/14/00	0.64	53	0.012	0.75	81	0.009	1.39	134	0.010		
8/21/00	0.35	30	0.012	0.77	87	0.009	1.12	117	0.010		
8/29/00	0.24	22	0.011	1.04	116	0.009	1.28	138	0.009		
9/5/00	0.59	55	0.011	0.45	31	0.015	1.04	86	0.012		
9/12/00	0.1	5	0.020	0.43	44	0.010	0.53	49	0.011		
9/19/00	0			0.73	94	0.008	0.73	94	0.008		
9/28/00	0.49	37	0.013	0.75	67	0.011	1.24	104	0.012		
10/3/00	0.46	39	0.012	1.65	144	0.011	2.11	183	0.012		
10/10/00	0				14		0	14			
10/16/00	0.81	65	0.012	2.72	297	0.009	3.53	362	0.010		
10/23/00	2.33	182	0.013	1.33	118	0.011	3.66	300	0.012		
10/31/00	9.51	648	0.015	0.99	103	0.010	10.5	751	0.014		
11/8/00	5.84	416	0.014	0.92	82	0.011	6.76	498	0.014		
11/14/00	1.42	97	0.015	3.21	251	0.013	4.63	348	0.013		
11/22/00	2.31	171	0.014	6.67	516	0.013	8.98	687	0.013		
11/28/00	0.7	51	0.014	3.25	277	0.012	3.95	328	0.012		
12/5/00	1.59	118	0.013	4.93	499	0.010	6.52	617	0.011		
12/12/00	0.15	9	0.017	3.12	330	0.009	3.27	339	0.010		
12/26/00	0.4	33	0.012	4.32	484	0.009	4.72	517	0.009		
3/1/99							4.46				
3/8/99							26.41				
3/15/99							20.4				
3/22/99							4.39	445	0.010	36	12.4

Appendix 3-1 (continued). Raw data for seasonal variation in *Z. texana* seed production.

Date	Sink wt	Sink no	Sink ave wt	Float wt	Float no	Float ave wt	Total wt	Total no	Total ave wt	No inflor	Seeds/inflor
3/29/99							27.77	1998	0.014	78	25.6
4/5/99	38.46	2658	0.013	6.79	679	0.010	45.25	3637	0.012	126	28.9
4/12/99	43.41	2894	0.015	5.37	597	0.009	48.78	3491	0.014	133	26.2
4/18/99	42.8	3057	0.014	5.46	606	0.009	48.26	3663	0.013	132	27.8
4/28/99	49.7	3313	0.015	12.3	946	0.013	62	4259	0.015	195	21.8
5/6/99	52.05	3470	0.015	7.78	707	0.011	59.83	4177	0.014	169	24.7
5/11/99	15.35	959	0.016	4.91	409	0.012	20.26	1368	0.015	115	11.9
5/17/99							17.65			129	
5/24/99	33.35	2223	0.015	2.99	344	0.009	36.34	2567	0.014	199	12.9
6/1/99	22.01	1572	0.014	3.33	333	0.010	25.34	1905	0.013	159	12.0
6/7/99	18.44	1085	0.017	5.92	538	0.011	24.36	1623	0.015	152	10.7
6/14/99	9.47	789	0.012	8.65	973	0.009	18.12	1762	0.010	147	12.0
6/21/99	6.89	626	0.011	7.18	798	0.009	14.07	1424	0.010	107	13.3
6/28/99	1.91	147	0.013	4.13	413	0.010	6.04	560	0.011	45	12.4
7/6/99	0.55	51	0.011	4.08	510	0.008	4.63	561	0.008	40	14.0
7/12/99	0.18	18	0.010	2.25	296	0.008	2.43	314	0.008	32	9.8
7/19/99	1.02	82	0.012	0.38	43	0.009	1.4	125	0.011	14	8.9
7/26/99	0.32	28	0.011	0.53	84	0.006	0.99	112	0.009	15	7.5
8/3/99	0.49	49	0.010	0.98	136	0.007	1.59	185	0.009	15	12.3
8/9/99	0.8	82	0.010	0.72	98	0.007	1.76	180	0.010	13	13.8
8/16/99	0.33	33	0.010	0.69	112	0.006	1.3	145	0.009	15	9.7
8/23/99	0.15	14	0.011	0.37	57	0.006	0.52	71	0.007	19	3.7
9/30/99	0.01	1	0.010	0.11	17	0.006	0.12	18	0.007	5	3.6
9/7/99	0.09	9	0.010	0.14	22	0.006	0.23	31	0.007	6	5.2
9/20/99	0.06	7	0.009	0.43	55	0.008	0.49	62	0.008	4	15.5
9/27/99	0.51	45	0.011	0.36	46	0.008	0.87	91	0.010	12	7.6
10/4/99	0.24	25	0.010	0.33	36	0.009	0.57	61	0.009	8	7.6
10/12/99	1.25	89	0.014	0.37	36	0.010	1.62	125	0.013	6	20.8

Appendix 3-1 (continued). Raw data for seasonal variation in *Z. texana* seed production.

Date	Sink wt	Sink no	Sink ave wt	Float wt	Float no	Float ave wt	Total wt	Total no	Total ave wt	No inflor	Seeds/inflor
10/18/99	0.61	44	0.014	0.41	44	0.009	1.02	88	0.012		
10/25/99	0.35	34	0.010	0.21	22	0.010	0.56	56	0.101	9	6.2
11/1/99	0.59	55	0.011	0.29	31	0.009	0.88	86	0.010	10	8.6
11/8/99	0.78	73	0.011	0.33	39	0.008	1.11	112	0.010		
11/15/99	1.35	100	0.014	0.94	90	0.010	2.29	190	0.012		
11/22/99	1.93	134	0.014	0.31	27	0.011	2.24	161	0.014		
11/29/99	0.95	71	0.013	0.36	34	0.011	1.31	105	0.012		
12/6/99	0.98	71	0.014	0.01	2	0.005	0.99	73	0.014		
12/14/99	0.34	19	0.018	0.21	23	0.009	0.55	42	0.013		

Appendix 4-1. *Zizania texana* seed quality study raw data. Fifty sinkers and 50 floaters were collected and weighed.

Collection Date	Treatment	Weight (g)	Collection Date	Treatment	Weight (g)
7/3/2003	sink	0.0072	7/3/2003	float	0.0073
	sink	0.0117		float	0.0078
	sink	0.0123		float	0.0053
	sink	0.0082		float	0.0049
	sink	0.0137		float	0.0050
	sink	0.0109		float	0.0075
	sink	0.0128		float	0.0063
	sink	0.0100		float	0.0063
	sink	0.0105		float	0.0058
	sink	0.0133		float	0.0142
	sink	0.0091		float	0.0059
	sink	0.0090		float	0.0068
	sink	0.0100		float	0.0049
	sink	0.0101		float	0.0068
	sink	0.0069		float	0.0064
	sink	0.0088		float	0.0060
	sink	0.0111		float	0.0073
	sink	0.0094		float	0.0085
	sink	0.0088		float	0.0065
	sink	0.0123		float	0.0057
	sink	0.0099		float	0.0072
	sink	0.0080		float	0.0068
	sink	0.0123		float	0.0079
	sink	0.0071		float	0.0059
	sink	0.0098		float	0.0073
	sink	0.0101		float	0.0057
	sink	0.0116		float	0.0052
	sink	0.0111		float	0.0043
	sink	0.0073		float	0.0060
	sink	0.0060		float	0.0065
	sink	0.0095		float	0.0046
	sink	0.0111		float	0.0061
	sink	0.0085		float	0.0067
	sink	0.0083		float	0.0071

Appendix 4-1 (continued). *Zizania seed* quality study raw data. Fifty sinkers and 50 floaters were collected and weighed.

Collection Date	Treatment	Weight (g)	Collection Date	Treatment	Weight (g)
7/3/2003	sink	0.0097	7/3/2003	float	0.0071
	sink	0.103		float	0.0056
	sink	0.107		float	0.0053
	sink	0.0086		float	0.0063
	sink	0.0067		float	0.0070
	sink	0.0079		float	0.0060
	sink	0.0090		float	0.0058
	sink	0.0083		float	0.0067
	sink	0.0088		float	0.0060
	sink	0.0112		float	0.0098
	sink	0.0084		float	0.0064
	sink	0.0086		float	0.0060
	sink	0.0072		float	0.0080
	sink	0.0106		float	0.0061
	sink	0.0122		float	0.0077
Total		0.4856	Total		0.3280
Mean		0.0097	Mean		0.00656
STDEV		0.0018	STDEV		0.00152
STD ERR		0.0003	STD ERR		0.0002

Appendix 4-2. Chi-square analysis of *Zizania texana* seedling development comparing development between sinkers and floaters. Categories included no chlorophyll, no roots, and chlorophyll and roots.

Treatment	Sinkers		Floaters	
	Observed	(f)	Observed	(f)
No chlorophyll	0.3	90.2	0.47	57.8
No roots	0.15	77.4	0.23	49.6
Chlorophyll and roots	0.36	203.5	0.55	130.5

$$\chi^2_{\text{test}} = 2.06$$

$$\chi^2_{\text{crit}} = 5.991 \text{ (2 DF)}$$

$$\chi^2_{\text{test}} < \chi^2_{\text{crit}} \text{ Accept } H_0$$

Appendix 5-1. Survey of *Zizania texana* reproductive activity in the San Marcos River. Data is ranked by distance from Cummins Dam. Note: Transects at 20 m intervals, first transect placement random; quadrat on 10 m interval along transect, first quadrat placement random. Study took place 17 May – 29 May, 2002. S.dev culms = submerged developing culms, E = emergent culms, SS = seed stage culms, CLP = clipped culms, SI = submerged inflorescence, and SHRD = shredded culms.

Date	Feet from Cummins Dam	Transect	Quadrat	Depth (m)	Velocity (m/s)	% Mat cover	S. dev culms	E	SS	CLP	SI	SHRD	Total
17-May	27,774	26	32	1.00	0.14	0	1	0	0	2	0	0	3
17-May	27,774	26	42	1.28	0.10	0	0	0	0	1	0	0	1
17-May	27,774	26	62	0.84	0.10	0	0	0	0	5	0	0	5
17-May	27,774	26	72	0.80	0.02	0	4	0	0	1	0	1	6
16-May	27,087	13	27	0.60	0.11	0	9	0	0	3	0	0	12
16-May	27,087	13	47	1.20	0.49	0	0	0	0	0	0	0	0
16-May	27,067	33	7	0.54	0.27	0	0	0	0	1	0	0	1
16-May	27,067	33	17	0.33	0.03	100	2	0	0	4	0	0	6
16-May	27,067	33	27	0.41	0.08	0	0	0	0	2	2	11	15
16-May	27,047	53	7	0.74	0.14	75	1	0	0	2	0	0	3
16-May	27,047	53	17	0.48	0.08	80	2	2	0	1	1	1	7
16-May	27,027	73	7	0.40	0.13	0	2	0	0	1	0	7	10
16-May	27,027	73	17	0.49	0.08	85	4	1	2	8	5	1	21
16-May	27,007	93	7	0.70	0.00	50	2	0	0	3	0	0	5
16-May	27,007	93	27	0.35	0.10	80	3	0	1	4	2	2	12
16-May	27,007	93	37	0.64	0.31	0	11	0	0	5	5	0	21
16-May	26,412	88	46	1.02	0.12	0	11	0	0	3	0	0	14
16-May	26,368	32	24	0.63	0.02	15	11	0	0	10	0	2	23
16-May	26,328	72	44	0.78	0.10	30	6	0	0	2	0	0	8

Appendix 5-1 (continued). Survey of *Zizania texana* reproductive activity in the San Marcos River. Data is ranked by distance from Cummins Dam. Note: Transects at 20 m intervals, first transect placement random; quadrat on 10 m interval along transect, first quadrat placement random. Study took place 17 May – 29 May, 2002. S.dev culms = submerged developing culms, E = emergent culms, SS = seed stage culms, CLP = clipped culms, SI = submerged inflorescence, and SHRD = shredded culms.

Date	Feet from Cummins Dam	Transect	Quadrat	Depth (m)	Velocity (m/s)	% Mat cover	S. dev culms	E	SS	CLP	SI	SHRD	Total
17-May	26,294	6	21	1.10	0.08	0	8	0	0	0	1	0	9
17-May	26,234	66	21	1.09	0.11	0	3	0	0	0	1	0	4
17-May	26,234	66	41	0.90	0.16	0	0	0	0	7	0	0	7
17-May	25,884	16	47	1.09	0.01	90	3	0	0	9	0	0	12
20-May	24,182	18	24	0.77	0.06	0	0	0	0	1	0	0	1
20-May	24,182	18	34	0.80	0.11	0	8	0	0	4	0	0	12
20-May	24,182	18	44	0.69	0.15	0	5	0	0	5	1	0	11
20-May	24,162	38	24	0.70	0.05	0	2	0	0	1	0	0	3
20-May	24,162	38	44	0.88	0.04	0	5	0	0	2	0	0	7
20-May	24,142	58	34	1.01	0.12	0	2	0	0	2	0	0	4
21-May	21,958	42	32	0.39	0.74	0	0	0	0	0	0	0	0
23-May	20,643	57	20	0.75	0.08	0	7	0	0	6	2	0	15
23-May	20,623	77	30	1.00	0.17	0	0	0	0	1	0	0	1
23-May	20,586	14	35	0.83	0.16	0	2	0	0	4	0	0	6
23-May	20,546	54	15	0.90	0.21	0	10	0	0	1	5	0	16
23-May	20,526	74	45	1.12	0.03	0	7	0	0	9	0	0	16
29-May	17,727	73	26	0.75	0.74	0	1	0	0	0	0	0	1
Total							132	3	3	110	25	25	298
Mean							3.67	0.08	0.08	3.06	0.69	0.69	8.28
SDev							3.63	0.37	0.37	2.80	1.45	2.16	6.35

Appendix 5-2. Survey of *Zizania texana* neighbors in the San Marcos River. Data are ranked by distance from Cummins Dam.

Date	Feet from Cummins Dam	Transect	Quadrat	Neighbors		River left	River Right
				Upstream	Downstream		
17-May	27,774	26	32	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
17-May	27,774	26	41	<i>Hygrophila polysperma</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
17-May	27,774	26	62	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
17-May	27,774	26	72	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
16-May	27,087	13	27	<i>Potamogeton illinoensis</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
16-May	27,087	13	47	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Potamogeton illinoensis</i>	<i>Zizania texana</i>
16-May	27,067	33	7	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Bare ground</i>	<i>Zizania texana</i>
16-May	27,067	33	17	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
16-May	27,067	33	27	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Potamogeton illinoensis</i>	<i>Zizania texana</i>
16-May	27,047	53	7	<i>Zizania texana</i>	<i>Potamogeton illinoensis</i>	<i>Potamogeton illinoensis</i>	<i>Zizania texana</i>
16-May	27,047	53	17	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
16-May	27,027	73	7	<i>Zizania texana</i>	<i>Hydrocotyle umbellata</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
16-May	27,027	73	17	<i>Potamogeton illinoensis</i>	<i>Zizania texana</i>	<i>Potamogeton illinoensis</i>	<i>Zizania texana</i>
16-May	27,007	93	7	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Bare ground</i>
16-May	27,007	93	27	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
16-May	27,007	93	37	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>

Appendix 5-2 (continued). Survey of *Zizania texana* neighbors in the San Marcos River. Data are ranked by distance from Cummins Dam.

Date	Feet from Cummins Dam	Transect	Quadrat	Neighbors		River left	River Right
				Upstream	Downstream		
16-May	26,412	88	46	<i>Potamogeton illinoensis</i>	<i>Zizania texana</i>	<i>Potamogeton illinoensis</i>	<i>Zizania texana</i>
16-May	26,368	32	24	<i>Hygrophila polysperma</i>	<i>Potamogeton illinoensis</i>	<i>Zizania texana</i>	<i>Potamogeton</i>
16-May	26,328	72	44	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Hygrophila polysperma</i>	<i>Hygrophila polysperma</i>
17-May	26,294	6	21	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
17-May	26,234	66	21	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
17-May	26,234	66	41	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
17-May	25,884	16	47	<i>Hygrophila polysperma</i>	<i>Ceratophyllum demersum</i>	<i>Potamogeton illinoensis</i>	<i>all three on all sides</i>
21-May	21,958	42	32	Bare ground	Bare ground	<i>Zizania texana</i>	Bare ground
23-May	20,643	57	20	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
23-May	20,623	77	30	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
23-May	20,586	14	35	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
23-May	20,546	54	15	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
23-May	20,526	74	45	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>	<i>Zizania texana</i>
29-May	17,727	73	26	Bare ground	Bare ground	Bare ground	Bare ground

Appendix 5-3. Biomass (g) of all *Zizania texana* structures after 20 weeks of growth in three different treatments: clipped, submerged, and control. Blanks indicate dead plants. Only five of the nine clipped plants were clipped, one died, and three did not produce flowering parts. * indicate plants that were in the clipped treatment but never developed emergent culms with awns to clip. This data was used in statistical analysis and in graphical analysis.

Treatment	Tillers	Culms	Vegetative	Above Root	Below Root	Culms & Tillers	Culms, Tillers & Above Root
submerged	19.54	14.04	1.52	6.88	26.73	33.58	40.46
submerged	9.11	13.26	5.25	4.41	15.79	22.37	26.78
submerged	5.62	9.93	14.22	1.2	6.59	15.55	16.75
submerged	21.3	20.97	9.22	4.44	15.49	42.27	46.71
submerged	16.05	14.44	1.65	5.39	12.18	30.49	35.88
submerged	18.82	24.93	5.51	7.14	10.59	43.75	50.89
submerged	8.24	9.66	16.42	1	6.96	17.9	18.9
submerged	3.07	39.29	14.77	3.96	20.51	42.36	46.32
submerged	-	-	-	-	-	-	-
Mean	12.7	18.3	8.57	4.3	14.3	31.0	35.3
STDEV	7.0	10.0	6.0	2.3	6.8	11.4	13.1
clipped	12.16	19.7	12.13	1.96	26.3	31.86	33.82
clipped	22.86	19.71	7.69	6.48	24.41	42.57	49.05
clipped	-	-	-	-	-	-	-
clipped*	21.79	9.13	4.14	0.69	8.14	30.92	31.61
clipped*	9.07	10.73	6.57	1.02	6.52	19.8	20.82
clipped*	0	2.31	20.8	0	15.03	2.31	2.31
clipped	0	1.7	23.3	7.73	19.08	1.7	9.43
clipped	7.81	-	-	1.76	-	-	1.76
clipped	14.9	6.74	12.86	4.34	11	21.64	25.98
Mean	11.1	10.0	12.5	3.0	15.8	21.5	21.8
STDEV	8.7	7.4	7.2	2.8	7.8	15.3	16.6
control	0	14.48	10.93	1.35	12.77	14.48	15.83
control	25.01	11.55	1.97	6.19	5.82	36.56	42.75
control	23.16	8.93	1.87	4.96	12.37	32.09	37.05
control	11.14	18.71	10.65	2.57	20.62	29.85	32.42
control	8.27	19.95	8.77	1.78	22.29	28.22	30
control	4.09	14.24	12.09	0.96	7.91	18.33	19.29
control	18.52	20.33	2.54	1.51	26.77	38.85	40.36
control	1.26	20.51	13.43	1.11	14.72	21.77	22.88
control	6.6	13.02	14.65	0.34	7.47	19.62	19.96
Mean	10.9	15.7	8.5	2.3	14.5	26.6	28.9
STDEV	9.3	4.3	5.1	2.0	7.3	8.5	9.9

Appendix 5-4. Raw data from *Z. texana* asexual reproduction greenhouse study.

Week	4		8		12		16		20	
Treatment	Culms	Tillers	Culms	Tillers	Culms	Tillers	Culms	Tillers	Culms	Tillers
Clipped	3	0	6	0	5	4	6	5	6	12
	0	0	0	0	0	0	0	0	1	0
	-	-	-	-	-	-	-	-	-	-
	4	1	6	2	5	4	7	3	7	10
	1	0	2	0	3	0	5	0	5	2
	1	0	6	0	5	5	6	8	8	10
	0	0	2	0	4	0	7	7	9	8
	2	0	6	0	5	5	3	8	7	11
	6	1	4	0	4	2	7	8	4	19
	Mean	2.1	0.3	4.0	0.3	3.9	2.5	5.1	4.9	5.9
	SDev	2.1	0.5	2.4	0.7	1.7	2.3	2.5	3.5	2.5
Control	7	7	6	8	9	9	12	14	6	7
	0	0	3	0	6	0	4	0	8	6
	6	0	6	2	8	5	6	18	8	29
	1	0	4	0	4	0	5	0	4	0
	2	0	9	0	14	0	13	0	14	0
	6	0	9	0	9	7	10	6	12	9
	2	0	3	1	6	2	8	3	9	6
	3	0	6	2	8	5	10	5	6	6
	0	0	3	0	4	0	4	0	5	3
	Mean	3.0	0.8	5.4	1.4	7.6	3.1	8.0	5.1	8.0
	SDev	2.7	2.3	2.4	2.6	3.1	3.5	3.4	6.7	3.3

Appendix 5-4 (continued). Raw data from *Z. texana* asexual reproduction greenhouse study.

Week	4		8		12		16		20	
Treatment	Culms	Tillers	Culms	Tillers	Culms	Treatment	Culms	Tillers	Culms	Tillers
Submerged	2	0	8	0	16	0	15	0	10	14
	9	1	12	2	11	1	13	3	13	9
	1	0	3	0	6	0	6	0	5	3
	6	0	9	0	7	0	10	0	12	6
	6	0	10	2	10	3	11	3	12	12
	1	0	2	0	2	0	2	0	2	1
	5	0	10	0	13	0	13	0	14	11
	6	0	16	0	10	0	14	1	12	1
	-	-	-	-	-	-	-	-	-	-
Mean	4.5	0.1	8.8	0.5	9.4	0.5	10.5	0.9	10.0	7.1
SDev	2.9	0.4	4.6	0.9	4.3	1.1	4.4	1.4	4.2	5.1

Appendix 6-1. Genetic Analysis of Texas Wild-Rice.

Richards, C.M., A. Reilley, D. Touchell, M.F. Antolin and C. Walters. Microsatellite primers for Texas wild rice, and a preliminary test of the impact of cryogenic storage on allele frequency at these loci. *Conservation Genetics*. (Accepted).

Richards, C.M., A. Reilley, M.F. Antolin and C. Walters. Genetic and demographic dynamics in *Zizania texana*: Conservation implication for an endangered aquatic plant. (to be submitted this summer)

Appendix 6-2. Protocols for Long-Term Seed Storage.

Walters, C, D.H. Touchell, P. Power, J. Wesley-Smith, and M.F. Antolin. 2002. A cryopreservation protocol for the embryos of the endangered species *Zizania texana*. Cryoletters 23:291-298.

Appendix 6-3. Vegetation Maps

Doyle, R.D. and R.M. Smart. 2000. Restoring Texas wildrice and other vegetation needed for fountain darters in the Comal and San Marcos Rivers. Progress Report 1/99 to 12/99. Interagency Number 1448-20181-97-N795.

Appendix 6-4. Monitoring Data from Wild Stands of Texas Wild-Rice.

Poole, J.M. 2002. Historical distribution of Texas wild-rice (*Zizania texana*) from 1989 to 2001. Section 6 final report. U.S. Fish and Wildlife Service, Albuquerque, New Mexico.

HISTORICAL DISTRIBUTION OF TEXAS WILD-RICE (*ZIZANIA TEXANA*) FROM 1989-2001

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INTRODUCTION: Texas wild-rice (*Zizania texana*) is an aquatic perennial grass (Poaceae) found only within the upper two miles of the San Marcos River (Figure 1). The area of its occurrence is almost entirely within the city limits of San Marcos in Hays County, Texas. This reach of the river, from its spring-fed headwaters to its confluence with the Blanco River, is remarkably clear and thermally constant. However cessation or diminution of spring flow, catastrophic flood events, several small dams, introduced non-native species, and the city of San Marcos with its associated recreation and pollution threaten the continued existence of Texas wild-rice. These and other threats led to the federal listing of this species (U.S. Fish and Wildlife Service 1978).

Historically Texas wild-rice was known from the upper San Marcos River, its irrigation canals, and Spring Lake (an impoundment at the spring headwaters) (Watkins 1930; Silveus 1933). By 1967 only one plant was known in Spring Lake and only scattered stands in the rest of the river (Emery 1967). The first quantified measurements of Texas wild-rice were done by Beaty who reported an aerial coverage of about 240 m² (Beaty 1975). In 1976 Emery produced the first map of the distribution of Texas wild-rice (Emery 1978). Emery also measured the coverage and calculated 1132.5 m² (Table 1; Emery 1977). Again he found no plants in Spring Lake, and scattered plants in the upper and lower segments of the upper two miles of the San Marcos River (Emery 1977; Emery 1978; Vaughan 1986). Although Emery measured coverage of wild-rice in 1978, he did not include the entire population as he had previously (Table 1; Vaughan 1986). Vaughan gathered coverage data using Emery's technique from 1983 to 1986 (Table 1; Vaughan 1986). Although the coverage fluctuated during those years, it was never higher than 541 m², a 50% decrease from Emery's 1976 data (Table 1; Vaughan 1986). Efforts to grow Texas wild-rice outside the San Marcos River have been mostly unsuccessful (Beaty 1976; Emery 1977; Terrell et al. 1978). A refugium collection has been established at the National Fish Hatchery and Technology Center in San Marcos and at the National Fish Hatchery in Uvalde, Texas. Several attempts to establish wild-rice within the San Marcos River or in cultivation in San Marcos have been successful (Beaty 1976; Emery 1977; Terrell et al. 1978; Vaughan 1986; Power 1996), although large-scale reintroduction projects have not been attempted.

Since 1989, Texas Parks and Wildlife (TPW) has been monitoring Texas wild-rice on an annual basis. Naturally occurring individual stands have been located using distance and bearing from witness points. Aerial coverage is figured on the length, width, and percent cover of each stand. Within established segments of the river, stand coverages are combined to produce a total for each segment. Data has been compiled in this manner in late June or early July from 1989 to 2001 (Table 1).

METHODS: Naturally occurring stands of Texas wild-rice have been precisely located and measured on an annual basis in late June or early July since 1989. A stand is defined as one or

more individual plants that are in physical contact. The location of each stand is measured from its upstream-most end in meters using a metric fiberglass tape, and in whole degrees (true north) using a Suunto sighting compass from a recorded reference point. During 1997 distances and bearings for stands in Segments A-G were measured using surveying equipment. During 1998 all segments were measured in this manner. In 1999 bearings were made using the surveying equipment, but distances were measured with the fiberglass tape. While surveying equipment is more accurate, the inherent difficulties with its use (training; difficult to maneuver in swift, deep water) made it less desirable. Although the tape and compass method is less accurate, it is much easier and less time-consuming. Originally witness points were either natural or cultural features, such as trees, concrete structures, etc. However the impermanence of these became obvious through time. A system of permanent, numbered, brass caps was installed and surveyed to centimeter accuracy in 1997 and 1998, and has been used to date.

To determine aerial coverage of stands, the length of the individual stands is measured from the upstream-most point of the stand to the downstream-most floating leaf tip. The width is measured at the widest point of the stand. This length and width create an imaginary rectangle that is used to determine the aerial coverage. Because the stand rarely occupies the entire rectangle, a percent cover is calculated. The percent cover is then multiplied by the length and the width to give an aerial coverage in square meters. Emery and Vaughan used a floating one meter quadrat to determine aerial coverage. However exactly how they used this is not known. In 1989 a floating one meter quadrat was used for the first few plants, but it was abandoned due to the difficulty of use and its time-consuming nature, both in the field and during data entry and calculation.

The river has been divided into segments that correspond primarily to cultural features (i.e., bridges, dams, etc.; see Figure 1). The original division was developed by Emery (Emery 1978), and was subsequently used by Vaughan (1986). Although TPW has changed the segment designations from numeric to alphabetic, the same segments are employed to facilitate comparisons between TPW data and that of Emery and Vaughan (Emery 1978; Vaughan 1986). Individual stands are identified by their alphabetic segment designation, and then numbered. Through the years, many new stands have been discovered and many stands have fragmented. To make stand accounting easier, stand fragments are given a small case alphabetic designation tagged on the parent stand alphanumeric code (for example, B2d). New stands, depending on their placement, are either given the next consecutive number (for stands downstream of others in the segment) or have a decimal number added to the designation of their closest neighbor (such as C7.1). During the course of the study a cumbersome alphanumeric system was employed. However while analyzing the data and mapping it in Arcview, it was decided to number the stands somewhat more logically. Appendix I lists all former stand designations by year, and what their equivalents are. The coverage of all stands within a segment is totaled to obtain coverage in square meters for the entire segment.

Data on stand number, distance, bearing, length, width, depth, and percent cover is entered into an Excel spreadsheet, and then through a specifically tailored software program attached by stand number to the reference point in an Arcview file. Thus all stands from all years can be shown graphically in reference to each other and to cultural and physical features on the landscape. Also by clicking on a stand, the entire history of that stand can be viewed. The

boundary of the river, the location of the reference points, and certain cultural features were produced using commercial surveying equipment. Thus their precision is at the centimeter level.

Written descriptions of stand locations were recorded for most stands beginning in 1990. In these descriptions, stand proximity to the bank and objects on or near the river banks were noted. When referring to the river bank, the left and right bank refer to the banks as looking downstream. Thus on the maps the bank on the left side or top of the page is actually the right bank. During the production of the maps, stands sometimes did not fall where they were known to have been from either previous or subsequent data. Usually this was just a reversed angle (all too easily done with a sighting compass). However on several occasions angles and/or distances from previous or subsequent years were employed to match the stand locations with descriptions. Although stands do not align perfectly through the years, the stands are dynamic. Also the program that attaches the stand rectangle to the reference point, always places the attachment point in the center of the upstream-most width of the plant. In the natural setting the upstream-most end is far from ever centered. Thus side-to-side shifts in stands may occur. Overall however these discrepancies have been overcome by comparing stand location and written descriptions through the years. Stands may overlap as percent cover within the rectangles is rarely 100%.

RESULTS: Thirteen years' worth of data have been compiled and analyzed. Appendix 2 includes all stand attributes (length, width, depth, percent cover, aerial coverage) segregated by year for the years 1989 through 2001. Yearly maps of stand location within the entire river are shown in Appendix 3. Annual changes in stand composition within segments (new, missing, reappearances, fragmentations, and coalescences as well as annual stand totals) are recorded in Appendix 4. In Appendix 5 individual stand placement and size at a larger scale is shown for smaller subsegments of the river. Finally Appendix 6 is a compilation of changes in aerial coverage for each stand from 1989 to 2001.

Below are segment descriptions that address the aerial coverage and stand changes within a given segment. When available, data from the studies of Emery and Vaughan (Emery 1978; Vaughan 1986) is included.

Segment A (Spring Lake Dam to University Drive): Neither Emery nor Vaughan recognized wild-rice as occurring in this segment (Emery 1978; Vaughan 1986). **In 1989** there were three stands in this segment, just above the University Drive bridge, with an aerial coverage of 23.1 m². **In 1990** a new stand appeared, bringing the total to four stands. Coverage increased dramatically as stands increased in size and became denser (i.e., higher percent cover). Aerial cover more than doubled (236%) to 77.63 m². **In 1991** stand number remained constant, and coverage increased slightly (just over 7%) to 83.39 m². **In 1992** stand number remained at four, and although one stand was not relocated, another stand was discovered. However coverage declined dramatically by almost 59% to 34.23 m² due to a decrease in the length and width of two stands. **In 1993** the stand missing in 1992 was relocated, bringing stand total back to five. Coverage increased (almost 13%) to 38.66 m². **In 1994** coverage decreased by over 10% to 34.43 m². Stand number increased by one to six stands total, with one new stand found, one stand producing two fragments, and two stands disappearing. **In 1995** coverage increased slightly by 4% to 35.93 m². Two new stands were discovered, and a small stand produced a small fragment. Total stand number was nine. **In 1996** coverage decreased by almost 12% to 31.7 m².

Three new stands found were offset by the loss of three stands. One stand coalesced with a fragment to yield a total of eight stands. **In 1997** 21 new stands appeared. Twenty of these stands appeared below the dam by the Spring Lake apartments where approximately 10,000 seeds were thrown into the river during the late summer of 1996. Although one small stand disappeared, total stand number was 28. There was an associated increase in coverage, almost 55%, to 48.99 m². **In 1998** four more new stands appeared, but three stands were lost. Four stands coalesced into two stands, and three stands coalesced into one. Total stand number was 25. Coverage continued to increase by just over 17% to 57.43 m². **In 1999** coverage increased by almost 32% to 75.77 m² and stand number increased to 29. The catastrophic fall flood of 1998 had little if any effect on this segment. Ten new stands were found, and one missing stand was relocated. One stand produced a fragment. Two stands coalesced, and seven stands disappeared. **In 2000** coverage barely increased (slightly over 2%) to 77.58 m². Stand number increased to 31, as two new stands were found and one was relocated. One stand produced another fragment, and two stands disappeared. **In 2001** coverage increased by almost 35% to the all-time high coverage of 104.41 m². However stand number decreased to 30. One new stand was found, but two stands were lost. Two stands fragmented, producing one fragment each. One stand coalesced with a fragment and another stand.

Segment B (University Drive bridge to Hopkins road railroad bridge): Like Segment A, neither Emery nor Vaughan recognized any wild-rice in this segment (Emery 1978; Vaughan 1986). **In 1989** coverage was 76.73 m² and there were 15 stands, all of which were between University Drive bridge and the first footbridge. **In 1990** coverage had more than doubled (almost 112%) to 162.44 m². Three new stands were found downstream of Sewell Park, and four new stands were discovered within the park. Four stands could not be relocated, and two stands coalesced into one. Total number of stands stood at 17. **In 1991** coverage increased again, by slightly over 46% to 237.8 m². Seven new stands appeared, and two stands reappeared. Two other lost stands appeared, but coalesced with a third stand. One stand fragmented, producing one fragment. One stand could not be relocated. Total number of stands increased to 26. **In 1992** coverage decreased by almost 13% to 207.72 m². Although four new stands were found, seven stands disappeared. One stand fragmented, producing one fragment, and one stand coalesced with its two fragments. Stand total was 22. **In 1993** coverage increased by almost 29% to 267.35 m². Twenty-four new stands appeared, and one stand reappeared. Only four stands disappeared. Three stands fragmented, two producing one fragment each, and one producing three fragments. Two stands coalesced, bringing the total to 47 stands. **In 1994** coverage increased by 56% to 417.17 m². Thirty-six new stands appeared, and two stands reappeared. Two stands fragmented: one producing two fragments, and one producing five fragments. One stand coalesced with one fragment, and another stand coalesced with two fragments. Two independent stands coalesced. Eight stands disappeared, bringing the total to 80 stands. **In 1995** coverage increased 23% to 513.07 m². Seventeen new stands were discovered, and one stand was relocated. Six stands fragmented with two producing one fragment each, one producing two fragments, two producing four fragments each, and one producing a record of eight fragments. Two independent stands coalesced, and three independent stands coalesced into one stand. Thirteen stands disappeared, bringing the total stand number to 102. **In 1996** coverage increased only slightly over 8% to 555.06 m². Twenty-five new stands were found, and one stand reappeared. Four stands fragmented, with three stands producing one fragment each, and one stand producing three fragments. Sixteen stands coalesced into eight stands, three stands coalesced into one, and five

stands coalesced into one. Twenty-four stands disappeared, for a total of 96 stands. **In 1997** coverage declined slightly by 6.4% to 519.68 m². Although 20 stands disappeared, 17 new stands were found and seven missing stands reappeared. In addition five stands fragmented: three producing one fragment each, one producing two fragments, and one producing four fragments. Six stands coalesced into three, four stands coalesced into one, and five stands coalesced into one. The overall total for this segment was 99 stands. **In 1998** coverage increased 47% to 766.5 m². Thirteen new stands appeared and six stands were relocated. Only five stands fragmented, each producing one fragment. Six stands became three stands, and three sets of three stands became one stand each. Eleven stands combined to form one giant stand. Only six stands disappeared, bringing the total number of stands to 98. **In 1999** after the catastrophic fall flood of 1998, coverage decreased by almost 14% to 661.81 m². Although coverage was down, 37 new stands were discovered and seven stands reappeared. Nine stands fragmented, with five stands producing one fragment each, three stands producing two fragments each, and one stand producing six fragments. Ten stands coalesced into one, four stands coalesced into one, and three stands also coalesced into one. Thirteen stands disappeared, giving a total of 132 stands. **In 2000** coverage increased by almost 13% to 745.09 m². Seventeen new stands appeared, and seven stands reappeared. Eleven stands fragmented. Seven stands produced one fragment each, two stands produced two fragments each, one stand produced three fragments, and one stand produced four fragments. Six stands coalesced into three, seven coalesced into one, three sets of four stands coalesced into one stand each, and six stands coalesced into one. Twenty-four stands disappeared, bringing the total to 127. **In 2001** coverage increased by 33% to an all-time high of 991.22 m². However total stand number decreased to 112, with 24 stands disappearing and 33 stands being lost to coalescence. Four stands coalesced into two stands. Three sets of three stands each coalesced into one stand. Nine stands coalesced into one, and 18 stands coalesced into one. Seven stands fragmented, with six producing one fragment each and one producing two fragments. Eight stands reappeared, and 26 new stands were found.

Segment C (Hopkins road railroad bridge to Rio Vista railroad bridge): **In 1976** Emery observed 37 clones with a coverage of 554 m² in this segment (Emery 1978). Coverage declined to 463.50 m² **in 1978**, and had continued to decline **in 1983** to 251 m², a decrease of over 45 % (Vaughan 1986). **In 1984** the decline was not as great (slightly over 9%), with coverage reduced to 228 m² (Vaughan 1986). **In 1985** coverage still fell, to 217 m², but the decrease was almost imperceptible, almost 5% (Vaughan 1986). **In 1986** the all-time low of 209 m² was reached, although the annual decline, almost 4%, was flattening out (Vaughan 1986). **In 1989** there were nine stands in this segment with an aerial coverage of 326.83 m². Stands C4, C5, C6, C7, C8, and C9 (see Appendix 4) matched the locations of some of Emery's mapped clones fairly well (Emery 1978). However in the case of stand C9, Emery (1978) indicated many more clones over a larger area than TPW observed in 1989. Emery (1978) also found five clones in the main channel below Snake Island, where stands were not found until 2000. **In 1990** coverage almost doubled, increasing by over 46% to 477.94 m², and number of stands almost doubled to 17. Six new stands were found, and three stands fragmented, producing one fragment each. One stand disappeared. **In 1991** coverage decreased about 22% to 392.02 m², and number of stands decreased to 13. Two stands disappeared, and two stands coalesced with their fragments. **In 1992** coverage increased about 15% to 449.23 m², while stand number decreased to nine. Four stands disappeared. **In 1993** coverage increased by slightly over 20% to 540.7 m², and number of stands increased to 13. One new stand appeared, and one stand was relocated. Two stands fragmented,

producing one fragment each. **In 1994** coverage decreased by slightly over 18% to 442.62 m², while number of stands increased to 14. Two new stands appeared, and one stand disappeared. **In 1995** coverage increased by slightly over 16% to 514.34 m², and stand number increased to 15. Two stands fragmented, one producing one fragment and the other producing two fragments. Two stands coalesced with one fragment each. One stand reappeared, and one stand disappeared. **In 1996** coverage decreased almost 11% to 459.95 m², and stand number also decreased to 14. One stand coalesced with two of its fragments, but also produced another fragment. Another stand also fragmented, producing one fragment. One stand disappeared. **In 1997** coverage dropped just under 10% to 416.09 m², and stand number increased to 15. One stand fragmented, producing three fragments. Another stand coalesced with one fragment, but produced another. Two stands disappeared. **In 1998** coverage increased very slightly (almost 2%) to 422.61 m², and stand number increased to 16. One stand coalesced with one fragment, but produced three more. One stand disappeared. **In 1999** in spite of the catastrophic 1998 fall flood, coverage increased by almost 17% to 493.08 m². However stand number decreased to 14. One new stand appeared, probably a plant that washed down from upstream. One stand coalesced with its fragment, and another stand coalesced with two fragments. **In 2000** coverage increased by slightly over 12% to 553.34 m², and number of stands increased to 19. Three new stands were found, while one stand disappeared. One stand coalesced with a fragment, and one stand produced another fragment. The largest stand, C5, produced three fragments. **In 2001** coverage declined by almost 28% to 399.16 m², but stand number only decreased by one, to 18. The largest stand, C5, coalesced with two fragments, but also produced two new fragments. Another stand coalesced with a fragment.

Segment D (Rio Vista railroad bridge to Rio Vista Dam): Neither Emery, Vaughan, nor TPW has ever found any wild-rice in this segment (Emery 1978; Vaughan 1986).

Segment E (Rio Vista Dam to low point on south side of Glover's Island): **In 1976** Emery observed 32 clones with a coverage of 55 m² (Emery 1978). Coverage dropped over 50% to 26 m² **in 1978** (Emery 1978). **In 1983** coverage increased slightly (by just over 11%) to 29 m² (Vaughan 1986). **In 1984** coverage decreased slightly (almost 7%) to 27 m² (Vaughan 1986). **In 1985** coverage decreased substantially (almost 30%) to 19 m², but coverage remained at this level **in 1986** (Vaughan 1986). **By 1989** coverage was back up to 81.33 m². Only 16 stands were observed in 1989 as compared to Emery's 32 from 1976 (Emery 1978). Emery (1978) found 13 clones above the Cheatham Street bridge where TPW found only five. The TPW stands were also closer to the bridge than most shown by Emery. In the part of the segment below the bridge, TPW found 10 stands in the area where Emery (1978) indicated 18 clones. There is exact correspondence between the last stand (TPW stand E14) in this segment, just above the segment break point, and a clone mapped by Emery (1978). **In 1990** coverage declined by just over 10% to 72.4 m², and stand number decreased to 15 with the coalescence of two stands. **In 1991** coverage increased by more than 50% to the all-time high of 109.81 m². However stand number actually decreased to 12 with the loss of two stands and the joining of two others. **In 1992** coverage declined about 35% to 71.86 m², but stand number increased to 15 with the discovery of two stands, the production of two fragments by one stand, and the loss of one stand. **In 1993** coverage increased slightly by just over 7% to 77.05 m², and stand number increased to 17. Six new stands were discovered, one stand reappeared, two stands coalesced, and four stands disappeared. **In 1994** coverage declined by just over 18% to 62.85 m², and stand number increased to 22. Three new stands appeared, and two stands reappeared. Two stands coalesced,

and two stands fragmented, producing two fragments each. Three stands disappeared. **In 1995** coverage increased almost 30% to 81.16 m², and stand number increased to 31. Two new stands were discovered. Three stands fragmented. One stand produced one fragment, and two stands produced four fragments each. Two stands disappeared. **In 1996** coverage decreased by about 10% to 72.83 m², and stand number decreased to 26. Two new stands appeared, and one stand reappeared. Three fragmented stands coalesced, including one with four fragments. One stand fragmented, producing one fragment. Three stands disappeared. **In 1997** coverage increased slightly by about 5% to 76.34 m²; however stand number decreased to 22. Three new stands appeared. Seven stands coalesced into three, and three stands disappeared. **In 1998** coverage decreased by about 11% to 67.75 m², but stand number increased to 32. Two new stands were discovered, and two stands reappeared. Four stands fragmented: two producing one fragment each, one producing three fragments, and one producing five fragments. Four stands disappeared. **In 1999** after the drastic fall flood of 1998, coverage decreased by almost 43% to 38.67 m², and stand number was cut in half to 16. Fifteen stands disappeared. Two stands fragmented: one producing one fragment and one producing two fragments. Seven stands coalesced into two stands (one stand coalesced with four fragments). One new stand was found. **In 2000** coverage declined again, by just over 37% to 24.25 m², and stand number decreased slightly to 14. Four stands disappeared, one new stand was found, and a stand reappeared. **In 2001** coverage continued to decline, by over 17% to the all-time low of 19.98 m². Stand number however increased to 19. Three new stands were found, and one stand reappeared. Three stands fragmented, producing one fragment each. Two stands disappeared.

Segment F: (low point on south side of Glover's Island to just above south I-35 access road): **In 1976** Emery observed five clones in the upper portion of Segment F, in the area occupied by stands F1 through F4 (Emery 1978). In the lower portion of Segment F, Emery identified 15 clones where one of the largest stands (TPW stand F12) in the river occurs (Emery 1978). However Emery (1978) found no clones between these two areas whereas TPW found several additional stands. Emery estimated coverage of 164 m² in this segment (Emery 1978). Emery did not provide coverage for this segment **in 1978** (Emery 1978). The reason for this is not known. When Vaughan began monitoring **in 1983** cover had dropped by over 25 % to 119 m² (Vaughan 1986). Cover dropped again (slightly over 30%) **in 1984** to the all-time low of 83 m² (Vaughan 1986). Coverage increased by almost 25% **in 1985** to 103 m², but decreased slightly over 10% to 92.5 m² **in 1986** (Vaughan 1986). **In 1989** coverage increased dramatically (almost 200%) to 276.57 m². While the increased coverage might be due to the difference in methodologies used by Emery/Vaughan and TPW, this was not a consistent pattern across segments. Although TPW found only 12 stands in this segment as compared to the 20 clones that Emery (1978) located, TPW found stands scattered throughout this segment (where Emery did not). Most of Emery's 15 clones in the lower end of the segment (Emery 1978) might be equivalent to the extremely large TPW stand F12. Thus the increase might represent an increase in clone/stand number as well as additional area occupied by Texas wild-rice (i.e., the middle part of the segment). **In 1990** three new stands were discovered below the large stand F12, one stand disappeared, and one stand fragmented in two, for a total of 15 stands. Coverage decreased slightly by about 13% to 241.9 m². **In 1991** coverage recouped its loss, gaining just over 12% to 271.42 m². Stand number remained constant, with three new stands being balanced out by two losses and two stands coalescing. **In 1992** coverage increased significantly (40%) to 380.08 m². Stand number also increased dramatically to 21 stands. Four new stands were discovered, two

stands reappeared, two stands fragmented (producing two fragments each), and four stands disappeared. **In 1993** coverage continued to increase slightly (just over 13%) to the all-time high coverage of 429.44 m². Stand number decreased to 20, with two new stands appearing, one stand reappearing, and six fragments coalescing into two stands. **In 1994** coverage declined steeply (37%) to 270.49 m². Although stand number only decreased by one to 19, four stands could not be relocated and two small stands coalesced. However these losses were weighed by three stands producing one fragment each and one new stand being discovered. **In 1995** coverage decreased almost imperceptibly (2%) to 276.28 m². Stand number decreased to 17 due to the coalescence of four stands into two, and the one stand lost balanced out by one new stand found. **In 1996** coverage remained essentially unchanged at 275.97 m². Stand number decreased to 15 with the loss of two stands. **In 1997** coverage increased by about 22 % to 335.4 m². Stand number increased to 16, with the relocation of two stands and the loss of one stand. **In 1998** coverage barely decreased by slightly over 2% to 327.6 m². However stand number increased to 21, primarily due to fragmentation (one stand produced one fragment, and one stand produced three fragments) but also to the discovery of a new stand. **In 1999** even with the drastic fall flood of 1998 that reduced coverage in several segments, coverage increased very slightly by almost 4% to 339.49 m². However stand number did decrease to 17, with the loss of nine stands and the fragmentation of three stands (two producing two fragments each and one stand producing one fragment). Also a stand and a fragment coalesced, and one new stand was found. **In 2000** coverage continued to increase slightly by just over 3% to 350.52 m², while stand number continued to decrease to 14. Three stands disappeared, two stands coalesced, one stand coalesced with two of its fragments, and two stands fragmented, producing one and two fragments each. **In 2001** coverage continued to just barely increase, by over 2% to 359.42 m². Stand number remained constant at 14 although one new stand was found, one stand reappeared, two stands with one fragment each coalesced, one stand fragmented producing one fragment, and one stand disappeared.

Segment G (just above the southbound access road of I-35 to Capes' Dam): **In 1976** Emery found 67.5 (68) m² and 29 clones (Emery 1978). Two of these appear to represent TPW stands G2 and G3, and probably TPW stand G1 is found among a cluster of plants upstream of the southbound access road. Interestingly Emery found eight plants above the southbound access road (where there have been one or two since), one plant between northbound I-35 and the northbound access road, four scattered plants between the northbound access road and Capes Dam, and 14 plants in the mill race canal just below the Capes Camp footbridge (Emery 1978). Since TPW began monitoring in 1989 no plants have been seen in the latter three areas (with the exception of one plant seen just below the northbound access in 1994 and 1995). Emery's **1978** monitoring showed that coverage declined over 51% to 33 m² (Vaughan 1986). Vaughan's monitoring showed that while the coverage increased by 12% to 37 m² **in 1983**, it declined sharply by 78% to 8 m² **in 1984**, and remained at this level **in 1985** (Vaughan 1986). **In 1986** coverage fell slightly by 6% to 7.5 m² (Vaughan 1986). When TPW began monitoring **in 1989**, coverage had more than doubled (148%) to 18.58 m². However this amount is still only about one-quarter of what Emery recorded in 1976, and only three stands remained as compared to Emery's 29 clones (Emery 1978; Vaughan 1986). The increase may be attributable to a change in methodology, especially in light of the fewer number of stands. **In 1990** coverage barely increased (1%) to 18.83 m², and stand number remained at three. **In 1991** coverage decreased by 32% to 12.88 m², as the three stands decreased in size. **In 1992** coverage decreased slightly (2%)

to 12.65 m², and stand number remained the same. **In 1993** coverage increased by 60% to 20.25 m², as the one of the three stands increased dramatically in size. **In 1994** coverage decreased again (13%) to 17.64 m², even though a new stand was found immediately downstream of I-35, bringing the total to four stands. The other three stands decreased in size. **In 1995** coverage continued to decline (16%) to 14.74 m², as one of the four stands decreased in size by half. **In 1996** coverage declined again (28%) to 10.64 m², as one stand decreased in size by half, and the stand below I-35 disappeared. **In 1997** coverage made a modest increase (13%) to 11.98 m², with the three stands increasing slightly in size. **In 1998** coverage increased dramatically (74%) to 20.8 m², as one of the three stands had a seven-fold increase in cover. **In 1999** coverage increased (12%) to 23.26 m², and two stands fragmented, producing one fragment each, making a total of five stands. Even one of the fragmented stands became dramatically smaller, another stand increased in size. **In 2000** coverage decreased (10%) to 20.85 m², even though the fragmented stands coalesced, bringing total stand number again to three. **In 2001** coverage decreased drastically by 77% to 4.78 m², the lowest coverage ever recorded. One stand disappeared, two stands fragmented (one producing one fragment and another producing three fragments), and one new stand appeared upstream. Total stand number stood at seven. The reason for the coverage decline and the disappearance and fragmentation of stands was due to a fallen tree that became lodged over the stands. Either by blocking light or by uprooting stands during its travel downstream, the stands were lost, reduced in size, or fragmented.

Segment H (Capes' Dam to an east-west channel through Thompson's Island): Emery only found one small plant in this segment **in 1976** with a coverage of only 0.5 m² (Emery 1978; Vaughan 1986). From his map it appears to be in the same general area as TPW stands H2 through H4. **In 1978** and during Vaughan's **1983-1986** monitoring (Vaughan 1986), no stands were relocated in this segment. However **in 1989** TPW located two stands in this segment: one very small stand not far below Cape's Dam and the other in the area indicated by Emery. Coverage was 11.4 m². **In 1990** coverage increased slightly (4%) to 11.82 m², and there was no change in stand number. **In 1991** coverage decreased by 27% to 8.66 m², due to the thinning of the larger stand. However two new small stands appeared, bringing stand total to four. **In 1992** coverage increased by 12 % to 9.74 m², even though the small stand below the dam could not be relocated, and the largest stand fragmented in two. Also one of the other smaller stands produced an even smaller fragment. However one of the small stands increased in size. Stand total was five. **In 1993** coverage decreased dramatically by 86% to 1.32 m². A very small piece of the stand below the dam was relocated. However the small stand and its fragment disappeared, and the stand that had increased in size the last year, became a very small stand once again. Although the largest stand coalesced with its fragment, another very small fragment was produced, and the stand was very thin with poor aerial coverage. Stand total dropped to four. **In 1994** coverage almost tripled (183%) to 3.73 m². A very tiny new plant was discovered just below Cape's Dam. However the other plant in the vicinity of the dam disappeared. Another small plant also disappeared downstream. Although the larger stand fragmented again, the larger stand became denser with a higher aerial coverage. Total number of stands remained at four. **In 1995** coverage increased again (35%) to 5.03 m². Although the tiny stand below the dam and one tiny fragment were lost, the remaining two stands increased slightly in size and fullness. **In 1996** coverage decreased by 14% to 4.35 m² due to a slight decrease in stand size. Although one fragment was lost, a new fragment was produced; thus the total remained at two stands. **In 1997** coverage decreased by 44% to 2.43 m². The main stand produced two more fragments, and itself became

much reduced in size and sparser. Total stand number was four. **In 1998** coverage increased by 17% to 2.84 m². A new stand appeared, bringing the total to five. **In 1999** coverage increased slightly (3%) to 2.92 m². The main stand produced two fragments, and became much reduced in size, bringing stand total to six. **In 2000** coverage increased by 26% to 3.67 m². Three stands coalesced into one much larger stand, and two very small stands coalesced into one small stand, bringing stand total to three. **In 2001** coverage decreased dramatically by 73% to 0.98 m² as the three small stands united to form a larger, but very sparse stand.

Segment I (east-west channel through Thompson's Island to Hays County Road): **In 1976**

Emery recorded six clones in the same general area as TPW stands I2-I8 (Emery 1978; Vaughan 1986). He did not show any clones in the vicinity of TPW stand I1, but he recorded five clones in an overflow channel from the Mill Race irrigation canal (Emery 1978). Total aerial coverage was 9 m² (Emery 1978; Vaughan 1986). For unknown reasons, Emery did not record any data in this segment in 1978 (Vaughan 1986). **In 1983** Vaughan recorded a coverage (4 m²) that was less than half (56%) that of Emery's (Vaughan 1986). **In 1984** coverage decreased again by 25% to 3 m² (Vaughan 1986). **In 1985** coverage increased by 50% to 4.5 m² (Vaughan 1986). At the end of Vaughan's study **in 1986** coverage was again 4 m², a decrease of 11% (Vaughan 1986). **By 1989** coverage was at an all-time high of 12.86 m² (an increase of over 200%), and seven stands were located. **In 1990** coverage decreased by 57% to 5.5 m², probably due to recreational trampling. One stand produced a fragment, and one new stand appeared, bringing stand total to 9. **In 1991** coverage decreased dramatically by almost 75% to 1.4 m². Four stands disappeared, bringing stand total to 5. Again this loss was probably due to recreational trampling. **In 1992** coverage decreased dramatically again by just over 85% to 0.21 m². Another four stands disappeared, bringing stand total to one. **In 1993** coverage increased by slightly over 52% to 0.32 m². One stand reappeared, bringing stand total to two. **In 1994** coverage decreased by almost 47% to 0.17 m². The stand that reappeared in 1993 disappeared for good, bringing stand total to one again. **In 1995** coverage continued its downward spiral to 0.11 m², a loss of slightly over 35%, as the one stand left continued to decrease in size. **In 1996** coverage decreased again, this time by almost 42%, to 0.064 m², as the one stand became just a few leaves. **In 1997** the stand finally disappeared, and no plants have been seen since in this segment.

Segment X (Hays County Road to mill dam, irrigation canal): Neither Emery nor Vaughan found Texas wild-rice in this area (Emery 1978; Vaughan 1986). However **in 1989** one stand with a coverage of 1.04 m² was located between the Hays County Road bridge and the mill dam. The stand disappeared by 1990.

Segment J (Hays County Road to just below confluence of natural and irrigation channels):

Emery mapped 39 clones with an aerial coverage of 49 m² in this segment of the river **in 1976** (Emery 1978; Vaughan 1986). His three clones in the upper part of the segment (Emery 1978) correspond with the two uppermost stands found by TPW in 1989. Emery (1978) shows three more clones at the first bend where TPW found one stand in 1989. In the area above the hatchery intake where TPW found four plants in 1989, Emery had indicated five clones in 1976 (Emery 1978). In the area of the hatchery intake and downstream to the confluence with the hatchery outfall, Emery showed 16 clones (Emery 1978). One of these was removed in the early 1980s for the intake structure, and the plant was given to researchers at Southwest Texas State University. By 1989 TPW found only five stands in this area. From below the confluence with the fish

hatchery outfall to the confluence of the main channel with the mill race channel, Emery located 13 clones, mostly at the lower end (Emery 1978). In 1989 TPW found 14 stands, but the stands were more evenly distributed along this stretch of the river. No data was reported by Emery **in 1978** (Vaughan 1986). Vaughan's aerial coverage **in 1983** was only slightly down (just slightly over 6%) to 46 m² from that of Emery's 1976 figures (Vaughan 1986). **In 1984** coverage decreased by slightly over 39% to 28 m² (Vaughan 1986). However **in 1985** coverage increased dramatically by almost 150% to 68 m² (Vaughan 1986). This increase was short-lived as coverage dropped again **in 1986** by almost 20% to 55 m² (Vaughan 1986). When TPW began monitoring **in 1989**, 27 stands with an aerial coverage of 95.03 m² were identified. Although two stands went missing, six stands coalesced into three stands, and one stand fragmented into two very small stands, coverage **in 1990** increased by almost 27% to the all-time high of 120.46 m². Stand total decreased to 23. Coverage decreased by slightly less than 3% to 117.01 m² **in 1991**, with three more stands missing and a new one discovered for a stand total of 21. Coverage remained almost identical **in 1992** at 117.39 m² (less than a 1% change), although two stands were lost and one stand fragmented, for a total of 20 stands. Coverage decreased by almost 18% to 96.57 m² **in 1993**. Although one stand was relocated and one stand reappeared, five stands disappeared and two stands produced one fragment each. Total stand number stood at 19. Coverage dropped again **in 1994**, this time by 21% to 76.22 m². One stand disappeared, but one stand reappeared. Thus stand total remained the same at 19. Coverage decreased by almost 39% to 46.58 m² **in 1995**. Five stands disappeared, four stands reappeared, five stands produced one fragment each, one stand produced two fragments, and one stand produced three fragments, for a stand total of 28. Coverage declined only 20% to 36.96 m² **in 1996**. Eight stands disappeared, three stands reappeared, one stand produced one fragment, and one stand produced two fragments, for a total of 26 stands. **In 1997** coverage remained essentially the same at 36.99 m² (less than 0.1%) even though nine stands disappeared. One new stand appeared and two missing fragments reappeared. One stand coalesced with its two fragments, and one stand coalesced with a fragment. Two stands fragmented, one producing one fragment and another producing two fragments. Stand total was 20. Coverage actually increased by almost 25% to 48.82 m² **in 1998**. One new stand appeared, and a missing stand reappeared. Two stands each coalesced with one fragment, and two independent stands coalesced into one. No stands disappeared, for a total stand number of 19. Coverage decreased by 85% to 7.33 m² **in 1999** due to the drastic flood of fall 1998. Twelve stands disappeared, one stand produced one fragment, two small stands appeared, and one fragment reappeared. Only 11 stands remained. **In 2000** coverage dropped another 15% to 6.22 m². Four more stands disappeared, one stand produced one fragment, a stand reappeared, and a new stand was found. Total stand number was 10. Coverage was up slightly by almost 8% to 6.7 m² **in 2001** even though three stands disappeared. One stand coalesced with its fragment, and a new stand was located, bringing the total number of stands to seven.

Segment K (just below confluence of natural and irrigation channels to just below transmission lines): **In 1976** Emery located 62 clones in K segment with a coverage of 233.5 m² (Emery 1978). For unknown reasons Emery did not record data for this segment **in 1978** (Vaughan 1986). However when Vaughan assessed this segment **in 1983**, coverage had dropped drastically (by over 76%) to 55 m² (Vaughan 1986). The decline was as dramatic **in 1984** when the coverage fell by almost 73% to 15 m² (Vaughan 1986). A striking rebound took place **in 1985** with coverage increasing by 363% to 69.5 m² (Vaughan 1986). Coverage remained almost

identical **in 1986** (only a 3.6% decrease) with 67 m² (Vaughan 1986). **In 1989** TPW found 45 stands in the river as compared to the 62 clones found by Emery in 1976 (Emery 1978). However the distribution of stands in K segment has changed dramatically since that time. In the upper 150 m of K segment, Emery found 18 clones in 1976 (Emery 1978) while TPW found 20 stands in 1989. In the next approximately 100 m river stretch, Emery located eight clones (Emery 1978) but only four stands were found by TPW in 1989. In the next 50 m river stretch, Emery found no clones (Emery 1978), but four stands were located in 1989 by TPW. In the next approximately 100 m, Emery found 20 stands (Emery 1978), but only eight stands were seen in 1989. In the next 50 m of the river, Emery saw three clones in 1976 (Emery 1978), but no stands were seen there in 1989. In the last 100 m of this segment, Emery found 13 stands (Emery 1978), and only nine stands were located there in 1989. A total aerial coverage of 77.14 m² was observed in 1989 by TPW which compares favorably with Vaughan's last total of 67 m² (Vaughan 1986) but not with Emery's 1976 amount of 233.5 m² (Emery 1978). **In 1990** nine new stands were discovered, four stands disappeared, and two stands fragmented, producing one fragment each, for a total of 52 stands. Coverage increased dramatically by about 150% to 191.02 m². **In 1991** three new stands were discovered, one stand reappeared, two stands coalesced into one, two stands produced one fragment each, and 11 stands disappeared, for a total of 46. Coverage decreased by about 10% to 171.52 m². **In 1992** two new stands were discovered, one stand reappeared, one fragmented stand coalesced, and five stands disappeared for a total of 43 stands. Coverage decreased by almost 30% to 122.56 m². **In 1993** three new stands were found, four stands reappeared, two stands grew together, three stands fragmented, two produced one fragment each and one stand produced two fragments, and 12 stands disappeared, for a total of 41 stands. Coverage increased slightly, up about 11% to 136.21 m². **In 1994** five new stands were located, two stands reappeared, a fragmented stand coalesced, two stands fragmented, one produced one fragment, and one produced two fragments, and five stands disappeared, for a total of 44 stands. Coverage decreased slightly by 5% to 129.5 m². **In 1995** two new stands were discovered, two stands reappeared, four stands fragmented (one stand produced one fragment, two stands produced two fragments each, and one stand produced three fragments), and seven disappeared, for a total of 49 stands. Coverage increased slightly by 5% to 136.24 m². **In 1996** one new stand was found, four stands reappeared, three stands coalesced (two stands with one fragment each, and one stand with two fragments), five stands fragmented (four stands produced one fragment each, and one stand produced two fragments), and eight stands disappeared, for a total of 48 stands. Coverage increased dramatically by almost 50% to 202.6 m². **In 1997** one stand reappeared, eight stands (four groups of two stands each) coalesced, one stand fragmented producing two fragments, and 16 stands disappeared, for a total of 31 stands. Coverage decreased by about 34% to 134.39 m². **In 1998** four new stands were found, seven stands reappeared, one stand and its two associated fragments coalesced, and four stands fragmented (two stands producing one fragment each, one stand producing two fragments, and one stand producing three fragments), for a total of 47 stands. Coverage increased dramatically by 75% to an all-time high of 234.9 m². **In 1999** after the devastating fall flood of 1998 two new stands were located, one stand fragmented producing one fragment, and 41 stands disappeared, leaving a total of nine stands. Coverage decreased drastically by almost 99% to an all-time low of 2.55 m². **In 2000** four new stands were discovered, one stand reappeared, and another two stands disappeared, for a total of 12 stands. Coverage almost tripled to 9.56 m². **In 2001** stand number remained constant at 12 stands while coverage decreased slightly by 6% to 8.97

Segment L (just below transmission lines to sewage treatment plant outfall): Neither Emery nor Vaughan reported any Texas wild-rice from the transmission lines to the sewage treatment plant (Emery 1978; Vaughan 1986). However Texas Parks and Wildlife found up to three stands in this area between 1989 and 1997. **In 1989** one stand was found just upstream of the sewage treatment plant with an aerial coverage of 2.84 m². **In 1990** the one stand decreased dramatically by almost 85% to 0.43 m². **In 1991** the one stand continued to decrease in size (almost 33%) to 0.29 m². **In 1992** the one stand increased in size (almost 14%) to 0.33 m². **In 1993** the one stand increased in size again (almost 58%) to 0.52 m². **In 1994** another stand was found farther upstream, bringing the total for this segment for two stands. Coverage increased to 1.52 m², an almost 200% increase. **In 1995** the upstream-most plant disappeared, leaving stand total at one plant. Coverage decreased by almost 200% back to 0.52 m². **In 1996** the missing stand reappeared, another stand appeared, and the stand nearest the sewage treatment disappeared, bringing stand total to two. Coverage increased by 275% to 1.95 m². **In 1997** the two upstream plants coalesced, leaving just one stand. Coverage decreased slightly (just over 4%) to 1.87 m². **In 1998** the stand disappeared.

Segment M (Sewage treatment plant outfall to Blanco River confluence): Emery reported one clone from approximately 400 m below the sewage treatment outfall on the north bank at the entrance to a 10 foot in diameter pipe (Emery 1976). Vaughan did not report any Texas wild-rice from below the sewage treatment plant (Vaughan 1986). In 1989 one plant was found downstream of the sewage treatment plant along the south bank near an ash in an opening near a major bend. The plant disappeared in 1990.

DISCUSSION: With the advent of GIS and software programs such as Arcview, it has become possible to precisely map the locations of objects. Although GPS equipment exists that allows mapping to centimeter accuracy, such equipment is prohibitively expensive (\$20,000+) and is not water-proof. Less costly GPS equipment (\$200-\$4,000, also not water-proof) is at best accurate to about one meter, and is usually in the three to five meter range. By using the system of permanent monument points surveyed to centimeter accuracy and considering a few centimeters from the stretch of a fiberglass tape, the distance would be less than 5 cm off. Normal bearing errors would be in the $\pm 2^\circ$ range. However the sighting compass is only as accurate as the user. Using a largely volunteer workforce it often happens that readings are not as accurate as possible. Also bearing errors increase with distance: for every degree of error, at a distance of 6 m, the point will be 10 cm off. Thus if the bearing is anywhere from 1 to 10 ° off, the point could be off 0.1 to 1 m at a distance of 6 m and as much 10 m at a distance of 60 m. Most wild-rice stands are within 30 m or less of the reference points so locations of stands, combining all possibilities for error, should be within 5 m or less. However from year to year map comparisons, most stands fell within one or two meters from their previous or subsequent years' positions.

All known collections of Texas wild-rice are from the upper San Marcos River, with the first specimen made in 1892. Although ecologically similar conditions occur in the headwaters of the Comal River in New Braunfels, Ferdinand Lindheimer, one of the first extensive plant collectors in Texas and resident of New Braunfels, never collected this species. When the species was first described (Hitchcock 1933), it was noted as being abundant in the San Marcos River, its irrigation canals, and Spring Lake (an impoundment at the river's spring source) (Watkins 1930;

Silveus 1933). By 1967 only one plant was located in Spring Lake, none in the uppermost 0.8 km of the river, only scattered stands in the lower 2.4 km, and none below this (Emery 1967). The first quantified measurements of Texas wild-rice were done by Beaty who reported about 240 m² (Beaty 1975). It is unfortunate that Beaty did not describe his survey methods or the area that he surveyed as his aerial coverage is substantially different from Emery's 1976 data (Emery 1978). In 1976 Emery produced the first map of the distribution of Texas wild-rice (Emery 1978). Emery also measured the coverage using a floating, one meter square frame, and calculated 1132.5 m² (Table 1; Emery 1977). This time he found no plants in Spring Lake, and scattered plants in the upper and lower segments of the upper two miles of the San Marcos River (Emery 1977; Emery 1978; Vaughan 1986). Although Emery measured coverage of wild-rice in 1978, he did not include the entire population as he had previously (Table 1; Vaughan 1986). Vaughan gathered coverage data using Emery's technique from 1983 to 1986 (Table 1; Vaughan 1986). Although the coverage fluctuated during those years, it was never higher than 541 m², a 50% decrease from Emery's 1976 data (Table 1; Vaughan 1986). On June 14, 1981, San Marcos received 13.98 inches of rain in a 24-hour period, the highest recorded rainfall between 1966 and 1990 (Bomar 1995). Probably this accounts for the dramatically lower aerial coverages between Emery's 1976 and Vaughan's 1983 data. Beginning in 1989 TPW recorded coverages up to triple those of Vaughan's 1986 data, plus coverage in several segments where neither Emery nor Vaughan had recorded wild-rice (see Table 1). Since 1989 coverage has increased with the exceptions of 1993-1994, 1996-1997, and 1998-1999. The reason for the decrease in coverage between 1993 and 1994 is unclear, but the severe drought during the summer and early fall of 1996 contributed to the decrease in coverage in 1997 and the catastrophic October 1998 flood extirpated most of the stands downriver of I-35. Although overall coverage for the entire natural population has increased, it obscures the fluctuations within segments and stands. For example the coverage in Segment B has increased almost 1200% (76.73 m² to 991.22 m²) since 1989 while Segments J and K dropped from highs of 120.46 m² and 234.9 m² to recent lows of 6.7 m² and 2.55 m². Thus it is important to analyze coverage changes by segment and in some cases by stand.

Neither Emery nor Vaughan recognized any Texas wild-rice in Segment A (Emery 1978; Vaughan 1986). Reasons for this are not known, although Southwest Texas State University may have dredged the bottom in this segment as they did immediately below University Drive bridge in Sewell Park. Aerial coverage in this segment increased from 1989 to 1991, but suffered an almost 60% decrease by 1992. One medium-sized stand disappeared, and two of the larger stands dramatically decreased in size (see Appendix 5). The reasons for this disappearance and decrease are not known, but these stands were in some of the swiftest water in the lower part of this segment. Although at a reduced level, coverage stayed fairly constant from 1992 to 1996. Sediment deposition from upstream construction on Sessoms Creek resulted in stand A3, its fragments, and nearby stands being slowly covered with sediment until all stands along the right bank just above University Drive bridge disappeared (see Appendix 5, maps for Segment A1:1989-2001). Since 1996 coverage has continuously increased. This is partially due to approximately 10,000 Texas wild-rice seeds being dispersed from the Spring Lake Dam downstream directly into this segment during the summer drought of 1996 (Kathryn Kennedy, pers. comm. 1998). In spring of 1997 dozens of small plants were noticed below the Spring Dam near the Clear Lake Apartments (Karim Aziz, pers. comm. 1997). During the 1997 summer monitoring 20 new wild-rice plants were recorded (see Appendix 5, maps A0: 1996 and 1997).

Other new plants have appeared in this upstream area since as well as a few downstream. Coverage has continued to increase in this segment despite the catastrophic fall flood of 1998 that had little effect on this segment. Damage to the Spring Lake Dam near Joe's Crabshack caused the entire segment to be fenced from late 1998 or early 1999 until after the summer 2000 monitoring. Thus there was less recreational impact. With the removal of the fence in 2001, no changes were noted from recreational impact, but the area in the lower segment from the middle to the right bank of the river should be carefully monitored for impacts. Segment A has gone from 7th in segment coverage in 1989 to 4th by 2001 (Table 1). There is much suitable habitat in this segment, although there is substantial impact from recreational trampling and sedimentation. In spite of most of the increase in coverage being due to an infusion of seeds that are progeny of plants from the I-35 bridge area, this segment is healthy, increasing, and now very important in the overall survival of wild-rice.

Like Segment A, neither Emery nor Vaughan recognized any Texas wild-rice in Segment B (Emery 1978; Vaughan 1986). Although aerial coverage was only at a moderate level in 1989, it has increased almost 13-fold (76.73 m² to 991.22 m²). Segment B contains more than half of all the Texas wild-rice found in the San Marcos River. It is also the most dynamic segment. Stands seen one month may be gone by the next. Number of stands has risen from a discrete 15 in 1989 to over 100, often difficult to distinguish, stands by 2001. Many of these stands have fragmented and coalesced several times. Coverage has risen more or less steadily from 1989 with a few setbacks: 1991-1992, 1996-1997, and 1998-1999. Between 1991 and 1992, several stands disappeared, and most stands became reduced in size. The reasons for this are not known. The decrease in coverage between 1996 and 1997 is due to the summer and fall drought of 1996 when water levels became too shallow for many stands. The decreased depth was also exacerbated by sediment deposition along the right bank just below the University Drive bridge, coming from upstream construction activities along Sessoms Creek, and by mats of vegetation and trash that cling to any above water vegetation. While the disastrous fall flood of 1998 did cause almost a 14% decrease in coverage, the number of stands actually increased (by 34 stands) with 37 new stands found, 7 relocated, and only 13 lost, in addition to fragmentation and coalescence. In 1989 wild-rice was only found between University Drive bridge and about 20 m above the first foot bridge (Appendix 5, map Segment B1-B15 1989). By 1990 a few plants had popped up in the City Park area (Appendix 5, maps Segment B16 1990 and Segment B17, B18 1990). The proliferation continued in 1991, with several more stands appearing in the City Park area (Appendix 5, map Segment B16.2-B18 1991). In 1992 the first plants below the first footbridge in Sewell Park appeared (Appendix 5, map Segments B15.2-B15.3 1992). By 1993 there were eight plants between the first and second footbridges in Sewell Park, and more plants appeared in the City Park area (Appendix 5, maps Segment B15.2-B15.10 1993 and Segment B16.3-B16.10 1993). In 1994 most of the new stands appeared in the uppermost part of the segment between University Drive and the first footbridge in Sewell Park (Appendix 5, map Segment B1-B15.13 1994). Additional new stands were found in 1995, but the more significant event was the first significant amount of fragmentation. Six stands produced a total of 20 fragments. This occurred almost exclusively in the upper segment, from mid-channel to left bank just above and below the first set of steps (Appendix 5: map Segment B1a-B15.13 1995). Recreation is high in this area, and may have been the cause. In 1996 the number of new stands (25) was just one more than the number of missing stands. There was also significant coalescence of stands, with 24 independent stands and/or fragments growing together to yield 11

distinct stands. This happened within the area of Sewell Park (Appendix 5: maps Segment B1a-B11.28 1996 and Segment B15.7-B15.34 1996). Also in 1996 stands were found for the first time below the Lions Club tube rental in City Park (Appendix 5: map Segment B20, B21 1996). 1997 was the first year for stand loss to exceed new stands found (20 vs. 17). However seven stands reappeared so stand gain still exceeded stand loss. The first mega-stand (called B10) developed this year, measuring 26.2 m X 9.4 m, and spanning the distances between the second and third right bank steps (Appendix 5: map Segment B1a-B14 1997). In general individual stand coverage was down in the upper segment between University Drive bridge and the first foot bridge due to the low flows caused by the 1996 drought. However several more stands were found in the City Park area below the Lions Club tube rental (Appendix 5: map Segment B22-27 1997) and the first stand appeared at the extreme end of Segment B, between Hopkins Street and the railroad bridge (Appendix 5: map Segment B28 1997). In 1998 the largest recorded stand (called B2) formed. It was almost 60 m long X 13.6 m wide, and stretched from University Drive bridge to midway between the second and third right bank steps in Sewell Park (Appendix 5: map Segment B1-B7e, B113-B11i 1998). More stands had appeared in Sewell Park between the first and second footbridges as well as in upper part of the City Park (Appendix 5: maps Segment 1998). Probably due to the catastrophic fall 1998 flood, in 1999 the super mega-stand fragmented although one mega-stand (B7) and several other large stands remained (Appendix 5: map Segment B1-B11.8 1999). In spite or perhaps because of the 1998 flood, stands continued to spring up, particularly in Sewell Park between the first and second footbridges (Appendix 5: map Segment B15.20-B15.10a 1999) and throughout City Park (Appendix 5: maps Segment B15.64-B16.26 1999, Segment B16.18-B16.23 1999, Segment B16.8-B17.9 1999, Segment B 17.4-B19 1999, Segment B21.1-27.1 1999, Segment B28, B29 1999, and Segment B30 1999). Although many stands were discovered or relocated, lost, fragmented, or coalesced in 2000, there were no significant changes, and coverage increased. Number of stands decreased in 2001 due to the coalescence of many stands into mega-stands B6 and B15.1 (see Appendix 5, maps Segment B1a-B11.29 2001 and Segment B15.1-B15.53 2001). Segment B went from 6th in segment coverage in 1989 to 1st by 1996, and by 2001 accounted for more than half of the wild-rice in the river (Table 1). Much suitable habitat exists in this segment, and the exponential growth of wild-rice here reinforces the quality of the habitat. However this segment of the river also has the highest amount recreational impact. Situated in Southwest Texas State University's Sewell Park and the city park of San Marcos, numerous recreationists including tubers, swimmers, canoeists, kayakers, scuba divers, snorklers, and others float, swim, paddle, walk, and handle wild-rice during the course of their activities. It is remarkable that wild-rice has withstood this pressure as well as it has. Also there are many floating vegetation/trash mats in this segment. The mats block light to wild-rice, eventually causing it to weaken and either die or become uprooted. Because this segment is now so important to the survival of the species, it is crucial that threats such as recreational impacts, sedimentation, and vegetation/trash mats be addressed.

Aerial coverage in Segment C declined dramatically from 1976 to 1986 (Vaughan 1986), but an overall increase in coverage began in 1989 (Table 1). However the coverage has fluctuated through the years. Coverage declined between 1990 and 1991 due to the thinning (as shown by reduced percent cover) of the large stands C5 and C6 (Appendix 4). The reasons for this are unknown, although sediment deposition began to cover the stand C6 area beginning in 1990. The coverage decline between 1993 and 1994 was due to a size decrease in the mega-stand C5. Reasons for this are not known (Appendices 4 and 6). The sediment is probably coming from

Purgatory Creek. A downward trend between 1995 and 1997 was due to the thinning and fragmentation of formerly large stands C6 and C9 (Appendices 4 and 6; Appendix 5: maps Segment C9 1996 and Segment C9-C9d 1997). The drought of 1996 may have had a deleterious effect on these stands. Also stand C6 is in shallow water, allowing recreational access from the adjacent private property. This may have had a detrimental effect on this stand. The fall flood of 1998 had little effect on coverage or stands in this segment. The decrease in coverage between 2000 and 2001 is due to the thinning of stands C2 and C5. Segment C contains one of the largest stands in the river. It also has good habitat for wild-rice, and has the second-highest segment coverage. However sedimentation, recreation (to a small extent), and at present a fallen tree (blocking light and presenting the possibility of uprooting wild-rice during flood events) threaten the continued growth and health of the plants in this segment.

Texas wild-rice has never been observed in Segment D (Emery 1978; Vaughan 1986). This segment is totally influenced by Rio Vista Dam which creates a deep pool and traps fine sediments (both conditions not conducive to healthy wild-rice growth).

Aerial coverage in Segment E dropped dramatically between 1976 and 1978, and remained low throughout Vaughan's study period (Emery 1978; Vaughan 1986). However when TPW began monitoring in 1989, coverage was up over 400%. Perhaps this was due to the change in methodology, but the segment's coverage did not increase comparatively across the board. This may represent a normal increase in cover over a period without floods, droughts, trampling, and other threats. Coverage in this segment was up and down between 1989 and 1998 (Table 1) due to the loss and gain of plants, as well as continual increases and decreases in coverage of individual stands. Coverage began a steady downward trend in 1999 after the drastic fall flood of 1998. The effect of the Capes Dam failure (early January 2000) resulted in a decrease in coverage, but the fall flood of 1998 was more drastic. Although plants were stressed along the shallow right bank in the middle of the segment below the Cheatham Street bridge, few plants disappeared. However several were reduced in coverage and undoubtedly stressed by the event. At present a large fallen tree is blocking light to several plants above the Cheatham Street bridge. Fortunately the tree is too large to get past the bridge during flood events, but the tree will trap additional debris, blocking more light. Recreational trampling has also been a problem in this segment. Many of the E6 cohort were in very shallow water easily accessible to humans and their pets. On several visits to this area, people and their dogs were observed playing in the wild-rice area. By 2001 all of the E6 group except for those in deeper water had disappeared (Appendix 5: maps Segment E6-E9.1 1999, Segment E6-E9.1 2000, and Segment E6-E9.1 2001). Although coverage has never been extremely high in this segment and has been somewhat variable through the years, the fall flood of 1998 and the breach of Cape's Dam in 2000 caused a downward spiral in coverage from which the segment has yet to recover. However much of the segment is extremely good habitat, and has high potential for recovery if threats such as recreation, erratic water levels, and tree falls are dealt with.

Aerial coverage in Segment F dropped by over 25 % between 1976 and 1984, and then was variable during the mid-1980s (Emery 1978; Vaughan 1986). The dramatic increase (almost 200%) in 1989 may have been due to differences in methodologies in estimating aerial coverage although these discrepancies are not consistent throughout. Also Emery (1978) reported clones only at the upper and lower end of the segment, whereas TPW found stands more or less

throughout the segment. Coverage continued to increase to an all-time high of 429.44 m² in 1993, only to drop by almost half in 1994 due to a similar decline in the three of the four largest stands (F6, F8, and F12) in this segment. Reasons for this decline are not known. Recreational impact in this segment is confined to two specific points. The one most impacted is at the I-35 bridge. Here an area of bare gravel exists where waders, swimmers, canoeists, anglers, and other recreationists have an easy, shallow water access to the river. Although no wild-rice has been seen in this area, it would provide suitable habitat. Although the 1998 fall flood had no discernible effect on the segment coverage, several plants (F3, F4, F4.4, and F5.1) were lost in the upper part of F segment (Appendix 5: maps Segment F3-F7 1998 and Segment F5-F7 1999) probably due to the raking of the bottom by a downed tree that moved downriver during the flood. During the breach of Cape's Dam in January 2000, stands F6 and F12 suffered major damage. Both stands came out of the water, particularly F12. A massive effort by USFWS and TPW removed many of the plants from primarily the F12 stand, and transferred them to the refuge at the National Fish Hatchery and Technology Center. When no room was left at the refuge, plants were planted in what appeared to be suitable habitat at the time. Although few plants survived the transplant in the river (these plants were the most stressed as they had been out of the water the longest), their contribution to 2000 annual coverage as well as the amazing recovery ability of wild-rice led to the small increase in segment coverage. Despite the 1998 fall flood and the breach in Capes Dam, this segment has increased in coverage and is at present holding steady. This segment had the 2nd highest segment coverage in 1989, but was out competed Segments A and C by 2001. However this segment is still extremely important to the continued survival of Texas wild-rice.

Compared to other segments, Segment G suffers from little impact due to recreation because of two factors: depth and private land ownership along most of the bank. The impact of I-35 is unknown, as the bridges have been there before Emery began his studies. The widening of the main lanes is considered to not have an impact, and several runoff and erosional concerns will be addressed by TxDOT during construction. Also USFWS has requested TxDOT to keep the area closed to vehicular traffic. This will lessen impact from recreation. Although Emery's all-time high coverage of 68 m² (Emery 1978; Vaughan 1986; Table 1) has never again been approached, coverage did recover from the lows of the Vaughan years (Vaughan 1986; Table 1). From 1989 to 2000, coverage more or less varied between 10 and 20 m² (Table 1). This segment is deep enough for plants not to have been affected by the breach in Cape's Dam, nor were they affected by the 1998 fall flood. However between 2000 and 2001 a fallen tree blocked sunlight to two stands, resulting in a drastic coverage loss when the tree was removed. Overall this segment is not a good segment for wild-rice. Cape's Dam backs up water to the I-35 bridge, providing little habitat for wild-rice.

Although Emery and Vaughan (Emery 1978; Vaughan 1986) found little if any wild-rice coverage in Segment H, TPW found a small amount. Coverage has been on a general decline in this segment since 1990 (Table 1). By 2001 only one plant remained, and aerial cover was less than one square meter. Neither drought nor the 1998 flood has had any visible effect on this segment. Reasons for its decline are unknown, although water clarity has decreased in all segments of the river below I-35. Segment H is not a premium habitat for wild-rice. Much of this segment is too deep, sluggish, and shaded for wild-rice.

Neither Emery nor Vaughan found more than 10 square meters of wild-rice in Segment I (Emery 1978; Vaughan 1986; Table 1). Aside from the all-time high coverage of 12.86 m² in 1989, coverage continuously dropped until wild-rice disappeared from this segment in 1997. As there is no aquatic vegetation in the lower portion of this segment (the area where the wild-rice occurred) and the area has high recreational traffic, it is highly likely that the recreational impact extirpated wild-rice in this segment. However there is some potentially suitable restorable habitat if recreation pressure can be alleviated in this segment.

Neither Emery nor Vaughan ever found any wild-rice in the irrigation channel between the county road bridge and the mill-race dam (Emery 1978; Vaughan 1986). In fact Emery never even gave this area a segment designation. Upon finding one stand in 1989, TPW designated this segment as X. However by the next monitoring session in 1990, the stand had disappeared for unknown reasons. No stands have since been seen in this segment, nor does it have suitable habitat for wild-rice.

Coverage in Segment J fluctuated during the Emery and Vaughan years (Emery 1978; Vaughan 1986), and increased the first two years of TPW's monitoring (Table 1). After 1990 coverage dropped almost continuously as stands thinned and fragmented. During 1998 fall flood 12 stands were lost (although one has reappeared), and segment cover was drastically reduced, reaching an all-time low of 6.22 m² in 2000. Although this segment shows no signs of recovery yet, Segment K, a segment similar in many ways to Segment J, made an impressive rebound between 1984 and 1990 (Table 1). Also the habitat remains quite suitable so restoration should be recommended for this segment.

From all-time highs of 233.5 m² in 1976 and 234.9 m² in 1998 to the all-time lows of 15 m² in 1984 and less than 10 m² in 1999-2001, coverage in Segment K has fluctuated violently over the last 25 years (Emery 1978; Vaughan 1986; Table 1). Emery located 62 clones in K segment in 1976 (Emery 1978) as compared to the 45 stands found in 1989, and the 13 stands left in 2001. The distribution of stands in K segment has also changed dramatically through time. Several stands in the middle of K segment (K21, K21.1, K22, K23, K24, K25, K26, K26.1, K27, K28, and K28.1) were ripped away by fallen trees as they moved downstream during various floods (Appendix 5: maps Segment K11-K21.1 1990, Segment K23 1990, Segment K25-K28.1 1990, Segment K11-K20 1991, Segment K26 1991, and Segments K23-K26.1 1992). The disappearance of several of these stands accounted for the lowered segment coverage in 1992. Likewise the loss of 16 stands between 1996 and 1997 caused a significant drop in coverage (Table 1). In the upper part of the segment, the left bank was stabilized with riprap sometime between the 1996 and 1997 monitoring sessions. Although the landowner and construction crew stayed away from the wild-rice, it is possible that sediment from the bank stabilization activities somehow disturbed the wild-rice. However stands were lost in the lower part of the segment as well which indicates that the bank stabilization may not have been at fault. Loss of the lower segment stands could not be tied to any specific cause. The 1998 fall flood did have a devastating effect on this segment. Coverage dropped from the all-time high of 234.94 m² to the all-time low of 2.55 m² (Table 1). Total number of stands went from 47 to 9 as 41 stands were lost (Appendix 4). Only one stand has reappeared in the ensuing two years, and no signs of recovery have been noted. However once before coverage in Segment K dropped a phenomenal amount. Between Emery's 1976 data and Vaughan's 1983 and 1984 data, coverage dropped from 233.5 m² to 15

m² (Table 1; Emery 1978; Vaughan 1986). Wild-rice recovered from that, and may yet again. There is much still suitable habitat within this segment, and it should be a high priority for reintroduction.

Neither Emery nor Vaughan reported any Texas wild-rice in Segment L, the area between the power lines and the sewage treatment plant (Emery 1978; Vaughan 1986; Table 1). Up to three plants were observed in this area by TPW between 1989 and 1997. However by 1998 all wild-rice has disappeared from this segment. As the plants were never very large (never more than 3 m²), they probably did not have the critical mass to maintain themselves. This segment does not have much habitat, as it is deep, murky, and shaded throughout much of the stretch.

Although Emery reported one plant below the sewage treatment outfall (Emery 1978), he did not give this segment an official designation or report any coverage for this plant. TPW called this Segment M, and found one plant in 1989 (although probably a different one from Emery's). The stand disappeared by 1990. Despite repeated trips through this segment as well as below the confluence with the Blanco River and on to Staples, no wild-rice has been observed by TPW. Most of the habitat above Cummings Dam (not far below the confluence with the Blanco River) is unsuitable for wild-rice as it is deep (over 2 m) and murky. Below Cummings Dam there appears to be suitable habitat (clear, fast flowing water no deeper than 2 m) but the river probably lacks the thermal constancy of the upper stretch.

Although overall coverage has increased since the Emery's 1976 data, the distribution of wild-rice has changed dramatically. Whereas wild-rice was scattered throughout the river, it is now almost exclusively limited to the section above the I-35 bridge. There is more impact in this area of the river from recreation, sedimentation, and pollution. Thus it is not a good scenario to depend on this upper reach for the continued existence of wild-rice. Healthy stands are needed in as much available suitable habitat in the upper San Marcos River (i.e., above the confluence with the Blanco River) as possible in order for this species to recover, if not survive.

CONCLUSIONS: The coverage and extent of Texas wild-rice has changed remarkably since the first reports in the 1930s of its abundance in the San Marcos River, associated irrigation canals, and Spring Lake. The differences between Vaughan's and Emery's data as well as the differences between their data and that collected by TPW for the last 13 years, highlights the fact that Texas wild-rice is capable of dramatic growth and recovery. However the same data show that the system is quite dynamic and able to cause overnight changes in the number of stands and coverage. As human mitigated threats such as flow diminution, recreation, introduction of non-native species, sedimentation, and pollution increase, wild-rice may be stretched beyond its limits of elasticity. There may simply come a point where there is not enough plant material left to recolonize areas of the river where wild-rice has been extirpated. Without the continued efforts of dedicated agency personnel and volunteers to maintain the quality and quantity of the San Marcos River as well as its constituent species such as Texas wild-rice, wild-rice may eventually reach the threshold from which there is no return, and wink out as many individual stands have already done.

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